OPI DATE 15/03/94 APPLN. ID 46916/93
AOJP DATE 09/06/94 PCT NUMBER PCT/AU93/00415

AU9346916

INT.....

(51) International Patent Classification 5 : C07K 7/08, C12N 15/... C12P 21/02, 21/08, G01N 33/68 A61K 39/36, 37/02	A1	(11) International Publication Number: WO 94/04564 (43) International Publication Date: 3 March 1994 (03.03.94)

(21) International Application Number: PCT/AU93/00415

(22) International Filing Date: 13 August 1993 (13.08.93)

(30) Priority data:
07/930,060 14 August 1992 (14.08.92) US

(71) Applicant *(for all designated States except US)*: THE UNIVERSITY OF MELBOURNE [AU/AU]; Grattan Street, Parkville, VIC 3052 (AU).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : KNOX, Robert, Bruce [AU/AU]; 9 Riverview Road, North Balwyn, VIC 3104 (AU). SINGH, Mohan, Bir [AU/AU]; 7 Lloyd Court, Templestowe, VIC 3106 (AU). ROLLAND, Jennifer [AU/AU]; 16 Tashinny Road, Toorak, VIC 3142 (AU). BLAHER, Bella [AU/AU]; 22 Carnarvon Road, North Caulfield, VIC 3161 (AU). SUPHIOGLU, Cenk [AU/AU]; 1/37 Proctor Cres., Keilor Downs, VIC 3038 (AU).

(74) Agents: SLATTERY, John, M. et al.; Davies Collison Cave, 1 Little Collins Street, Melbourne, VIC 3000 (AU).

(81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: T CELL EPITOPES OF RYEGRASS POLLEN ALLERGEN

(57) Abstract

The present invention provides isolated peptides of *Lol p* V, a major protein allergen of the species *Lolium perenne*. Therapeutic peptides within the scope of the invention comprise at least on T cell epitope, or preferably at least two T cell epitopes of a protein allergen of *Lol p* V. Diagnostic peptides within the scope of the invention bind IgE. The invention also provides modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects. The invention further provides nucleic acid sequences coding for peptides of the invention. Methods of treatment or diagnosis of sensitivity to *Lol p* V or an allergen immunologically related to *Lol p* V in an individual. Therapeutic compositions comprising one or more peptides of the invention are also provided.

T CELL EPITOPES OF RYEGRASS POLLEN ALLERGEN

Background of the Invention:

Allergens constitute the most abundant proteins of grass pollen, which is the major cause of allergic disease in temperate climates (Marsh (1975) Allergens and the genetics of allergy: in M. Sela (ed). The Antigens. Vol. 3, pp 271-359. Academic Press Inc., London, New York).. Hill et al. (1979) Medical Journal of Australia 1, 426-429). The first descriptions of the allergenic proteins in ryegrass showed that they are immunochemically distinct, and are known as groups I, II, III and IV (Johnson and Marsh (1965) Nature, 206, 935-942; and Johnson and Marsh (1966) Immunochemistry 3, 91-100). Using the International Union of Immunological Societies' (IUIS) nomenclature, these allergens are designated *Lol p I*, *Lol p* II, *Lol p* III and *Lol p* IV. In addition, another important *Lolium prenne L.* allergen which has been identified in the literature is *Lol p V* also known as *Lol p IX* or *Lol p Ib* (Singh et al. (1991) *Proc. Natl. Acad. Sci. USA*. 88:1384-1388. Suphioglu, et al. 1992. Lancet. 339: 569-572.

These five proteins have been identified in pollen ryegrass, *Lolium perenne* L., and act as allergens in triggering immediate (Type 1) hypersensitivity in susceptible humans.

Lol p V is defined as an allergen because of its ability to bind to specific IgE in sera of ryegrass-sensitive patients, to act as an antigen in IgG responses and to trigger T-cell responses. The allergenic properties have been demonstrated by immunoblotting studies showing 80% of ryegrass pollen sensitive patients possessed specific IgE antibody that bound to *Lol p* V isoforms (PCT applicaton publication no. WO 93/04174, page 65). These results indicate that *Lol p V* is a major ryegrass allergen.

Substantial allergenic cross-reactivity between grass pollens has been demonstrated using an IgE-binding assay, the radioallergo-sorbent test (RAST), for example, as described by Marsh et al. (1970) J. Allergy, 46, 107-121, and Lowenstein (1978) Prog. Allergy, 25, 1-62. (Karger, Basel).

The immunochemical relationship of *Lol p* V with other grass pollen antigens have been demonstrated using both polyclonal and monoclonal

antibodies (Zhang et al., *Int Arch Allergy Appl Immunol*. 96:28-34 (1991); Roberts et al., *Int Arch Allergy Appl Immunol*. 98:178-180 (1992); and Mattheisen and Lowenstein. *Clinical and Experimental Allergy*. 21:309-320 (1991). Antibodies have been prepared to purified proteins that bind IgE components. These data demonstrate that a major allergen is present in pollen of closely related grasses is immunochemically similar to *Lol p* V and are generally characterized as Group V allergens.

Summary of the Invention

The present invention provides isolated peptides of *Lol p* V. Peptides to be used therapeutically within the scope of the invention comprise at least one T cell epitope, preferably at least two T cell epitopes of *Lol p* V. The invention further provides peptides comprising at least two regions, each region comprising at least one T cell epitope of *Lol p* V. Peptides of the invention to be used for diagnostic purposes are capable of binding IgE and comprise at least one B cell epitope.

The invention also provides modified peptides having similar or enhanced therapeutic properties as the corresponding, naturally-occurring allergen or portion thereof, but having reduced side effects, as well as modified peptides having improved properties such as increased solubility and stability. Therapeutic peptides of the invention are capable of modifying, in a *Lol p* V-sensitive individual to whom they are administered, the allergic response of the individual to *Lol p* V or an allergen immunologically cross-reactive *Lol p* V.

Methods of treatment or of diagnosis of sensitivity to grass allergen in an individual and therapeutic compositions comprising one or more peptides of the invention are also provided.

The present invention also provides derivatives or homologues of *Lol p* V peptides and peptides immunologically cross-reactive to antibodies to *Lol p* V or immunologically cross-reactive with T cells of *Lol p* V or derivatives or homologues thereof.

Further features of the present invention will be better understood from the following detailed description of the preferred embodiments of the invention in conjunction with the appended figures.

Brief Description of the Figures

Fig. 1 shows the nucleotide sequence of cDNA clone 12R (SEQ ID NO:1) and its predicted amino acid sequence (SEQ ID NO:2). Clone 12R is a full-length clone of *Lol p* V.

Fig. 2 shows various peptides of the invention of various lengths derived from *Lol p* V.

Fig. 3 is a graphic representation of the stimulation index of T cells from day 34 of the T cell line cultured with autologous irradiated PBMC in the presence of the inducing allergen *Lol p* V, crude ryegrass pollen extract or crude Bermuda grass pollen extract; proliferation as assessed by [3H]TdR incorporation, the results are expressed as the stimulation index, determined by dividing the average CPM of cells with allergen by the average CPM of cells with medium, alone, each allergen concentration was tested in triplicate.

Fig. 4 is graphic representation of the proliferative response of a T cell line derived from a healthy ryegrass pollen allergic adult, on day 49 to the *Lol p* V synthetic peptides, the results are expressed as the average counts per minute (CPM) of duplicate cultures, background response of cells in the absence of allergen was 4849 CPM.

Fig. 5 is a graphic representation of the proliferative response of T cell clone A12 to the *Lol p* V synthetic peptides, the results are expressed as the average counts per minute (CPM) of duplicate cultures, background response of cells in the absence of allerge was 358 CPM, and the Y-axis is shown on a log scale.

Fig. 6 is a graphic representation of the proliferative response of a second T cell line derived from a healthy ryegrass pollen allergic adult, to the *Lol p* V synthetic peptides, the results are expressed as the average counts per minute (CPM), the background response of cells in the absence of allergen was 2500 CPM, and the concentration of peptide was 0.1 µg/ml.

Fig. 7 shows a dot blot immunoassay of the 35 overlapping peptides from *Lol p* V, (clone 12R) immobilized on nitrocellulose (NC) filter and

screened with individual sera showing human IgE binding. mAbs FMC-A7 and polyclonal anti-*Lol p* V rabbit antibody. C represents crude ryegrass pollen extract (1µg/dot) used as a positive control.

Fig. 8 shows identification of B cell epitopes of *Lol p* V based on dot immonoassays of overlapping sequential synthetic peptides derived from clone 12R: Fig. 8a shows the IgE binding from 16 positive sera from a total of 50 ryegrass pollen allergeic patients (Rast≥4); and Fig. 8b shows monoclonal or polyclonal antibody binding, the values are arbitrary densitometric units of the intensity of the dot blot, measured by Pharmacia LKB UltraScan XL, Sweden. densometric values ≥2 were considered as positive binding in comparison to the background, for every peptide, the sera and antibody values have been added (score/peptide) and divided by the number of positive sera or antibody to express the final value as an average (score/sera or antibody), sera C1 and C2 were used as negative controls (C refers to crude ryegrass pollen extract (1 µg/dot) as a positive control).

Fig. 9 shows an inhibition peptide dot-blot with peptide 34 (SEQ ID NO:36), peptides 6 (SEQ ID NO:8), 24 (SEQ ID NO:26), and 31 (SEQ ID NO:33) were used as negative controls; FPLC purified Lol p V designated as "IX" and crude ryegrass pollen extract designated as "C" were used as positive controls.

Fig. 10 is a graphic depiction of an Inhibition ELISA with peptide 34 (SEQ ID NO:36), peptides 6 (SEQ ID NO:8), 24 (SEQ ID NO:26), and 31 (SEQ ID NO:33) were used as negative controls; and crude ryegrass pollen extract was used as a positive control.

Detailed Description of the Invention

The present invention provides isolated peptides derived from *Lol p* V. As used herein, a peptide or fragment of a protein refers to an amino acid sequence having fewer amino acid residues than the entire amino acid sequence of the protein. The terms "isolated" and "purified" as used herein refer to peptides of the invention which are substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or substantially free of chemical precursors or other chemicals when synthesized chemically. As used herein, the term "peptide" of the invention

include peptides derived from *Lol p* V which comprise at least one T cell epitope of the allergen or a portion of such peptide which comprises at least one T cell epitope and/or peptides derived from *Lol p* V which binds IgE and which comprise at least one B cell epitope.

Peptides comprising at least two regions, each region comprising at least one T cell epitope of *Lol p* V are also within the scope of the invention. Isolated peptides or regions of isolated peptides, each comprising at least two T cell epitopes of *Lol p V* protein allergen are particularly desirable for increased therapeutic effectiveness. Peptides which are immunologically related (e.g., by antibody or T cell cross-reactivity) to peptides of the present invention are also within the scope of the invention. Peptides immunologically related by antibody cross-reactivity are bound by antibodies specific for a peptide of *Lol p* V Peptides immunologically related by T cell cross-reactivity are capable of reacting with the same T cells as a peptide of the invention.

Isolated peptides of the invention can be produced by recombinant DNA techniques in a host cell transformed with a nucleic acid having a sequence encoding such peptide. The isolated peptides of the invention can also be produced by chemical synthesis. When a peptide is produced by recombinant techniques, host cells transformed with a nucleic acid having a sequence encoding a peptide of the invention the functional equivalent of the nucleic acid sequence are cultured in a medium suitable for the cells and peptides can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis or immunopurification with antibodies specific for the peptide, the protein allergen from which the peptide is derived, or a portion thereof.

The present invention provides expression vectors and host cells transformed to express the nucleic acid sequences of the invention. Nucleic acid coding for a *Lol p V* peptide of the invention or at least one fragment thereof may be expressed in bacterial cells such as *E. coli*, insect cells, yeast, or mammalian cells such as Chinese hamster ovary cells (CHO). Suitable expression vectors, promoters, enhancers, and other expression control elements may be found in Sambrook et al. *Molecular Cloning: A Laboratory*

Manual, second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. Other suitable expression vectors, promoters, enhancers, and other expression elements are known to those skilled in the art. Suitable vectors for expression in yeast include YepSec1 (Baldari et al. (1987) *Embo J.* 6: 229-234); pMFa (Kurjan and Herskowitz (1982) *Cell* 30: 933-943); JRY88 (Schultz et al. (1987) *Gene* 54: 113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). These vectors are freely available. Baculovirus and mammalian expression systems are also available. For example, a baculovirus system is commercially available (PharMingen, San Diego, CA) for expression in insect cells while the pMSG vector is commercially available (Pharmacia, Piscataway, NJ) for expression in mammalian cells.

For expression in *E. coli*, suitable expression vectors include, among others, pTRC (Amann et al. (1988) *Gene* 69: 301-315); pGEX (Amrad Corp., Melbourne, Australia); pMAL (N.E. Biolabs, Beverly, MA); pRIT5 (Pharmacia, Piscataway, NJ); pET-11d (Novagen, Madison, WI) Jameel et al., (1990) *J. Virol.* 64:3963-3966; and pSEM (Knapp et al. (1990) *BioTechniques* 8: 280-281). The use of pTRC, and pET-11d, for example, will lead to the expression of unfused protein. The use of pMAL, pRIT5 pSEM and pGEX will lead to the expression of allergen fused to maltose E binding protein (pMAL), protein A (pRIT5), truncated ß-galactosidase (PSEM), or glutathione S-transferase (pGEX). When a *Lol p* V peptide of the invention is expressed as a fusion protein, it is particularly advantageous to introduce an enzymatic cleavage site at the fusion junction between the carrier protein and *Lol p* V peptide. The *Lol p* V peptide may then be recovered from the fusion protein through enzymatic cleavage at the enzymatic site and biochemical purification using conventional techniques for purification of proteins and peptides. Suitable enzymatic cleavage sites include those for blood clotting Factor Xa or thrombin for which the appropriate enzymes and protocols for cleavage are commercially available from, for example, Sigma Chemical Company, St. Louis, MO and N.E. Biolabs, Beverly, MA. The different vectors also have different promoter regions allowing constitutive or inducible expression with, for example, IPTG induction (PRTC, Amann et al., (1988) *supra*; pET-11d, Novagen, Madison, WI) or temperature induction (pRIT5, Pharmacia, Piscataway, NJ). It may also be appropriate to express

recombinant *Lol p* V peptides in different *E. coli* hosts that have an altered capacity to degrade recombinantly expressed proteins (e.g. U.S. patent 4,758,512). Alternatively, it may be advantageous to alter the nucleic acid sequence to use codons preferentially utilized by E. coli, where such nucleic acid alteration would not affect the amino acid sequence of the expressed protein.

Host cells can be transformed to express the nucleic acid sequences of the invention using conventional techniques such as calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, or electroporation. Suitable methods for transforming the host cells may be found in Sambrook et al. *supra*, and other laboratory textbooks. The nucleic acid sequences of the invention may also be chemically synthesized using standard techniques (i.e. solid phase synthesis). Details of the isolation and cloning of clone 12R encoding *Lol p* V (described as *Lol p* Ib.1) are given in PCT application Publication Number WO 93/04174.

The present invention also provides nucleic acid sequences encoding peptides of the invention. Nucleic acid sequences used in any embodiment of this invention can be cDNAs encoding the corresponding peptide sequences as shown in Fig. 7, or alternatively, can be any oligodeoxynucleotide sequence having all or a portion of a sequence represented herein, or their functional equivalents. Such oligodeoxynucleotide sequences can be produced chemically or mechanically, using known techniques. A functional equivalent of an oligonucleotide sequence is one which is 1) a sequence capable of hybridizing to a complementary oligonucleotide to which the sequence (or corresponding sequence portions) of *Lol p* V as shown in Fig. 1 (SEQ ID NO:1) or fragments thereof hybridizes, or 2) (the corresponding sequence portions complementary to the nucleic acid sequences encoding the peptide sequence derived from *Lol p* V as shown in Table 1 and Fig. 2 (SEQ ID NO:1), and/or Fig. 3) a sequence which encodes a product (e.g., a polypeptide or peptide) having the same functional characteristics of the product encoded by the sequence (or corresponding sequence portion) of *Lol p* V as shown in Fig. 1 (SEQ ID NO:1). Whether a functional equivalent must meet one or both criteria will depend on its use (e.g., if it is to be used only as an oligoprobe, it need meet only the first or second criteria and if it is to be used

to produce a *Lol p V* peptide of the invention. it need only meet the third criterion).

The present invention also provides a method of producing isolated *Lol p* V peptides of the invention or a portion thereof comprising the steps of culturing a host cell transformed with a nucleic acid sequence encoding a *Lol p* V peptide of the invention in an appropriate medium to produce a mixture of cells and medium containing said *Lol p* V peptide; and purifying the mixture to produce substantially pure *Lol p* V peptide. Host cells transformed with an expression vector containing DNA coding for a *Lol p* V peptide of the invention or a portion thereof are cultured in a suitable medium for the host cell. *Lol p* V peptides of the invention can be purified from cell culture medium, host cells, or both using techniques known in the art for purifying peptides and proteins including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis and immunopurification with antibodies specific for the *Lol p* V peptides or portions thereof of the invention.

Another aspect of the present invention pertains to an antibody specifically reactive with a *Lol p* V peptide. Such antibodies may be used to standardize allergen extracts or to isolate the naturally occurring *Lol p* V. Also, *Lol p* V peptides of the invention can be used as "purified" allergens to standardize allergen extracts. For example, an animal such as a mouse or rabbit can be immunized with an immunogenic form of an isolated *Lol p* V peptide of the invention capable of eliciting an antibody response. Techniques for conferring immunogenicity on a peptide include conjugation to carriers or other techniques well-known in the art. The *Lol p* V peptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum standard ELISA or other immunoassay can be used with the immunogen as antigen to assess the levels of antibodies.

Following immunization, anti-*Lol p* V peptide antisera can be obtained and, if desired, polyclonal anti-*Lol p* V peptide antibodies from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield

hybridoma cells. Hybridoma cells can be screened immunochemically for production of antibodies reactive with the *Lol p* V peptides of the invention. These sera or monoclonal antibodies can be used to standardize allergen extracts.

Through use of the peptides and antibodies of the present invention, preparations of consistent, well-defined composition and biological activity can be made and administered for therapeutic purposes (e.g. to modify the allergic response of a ryegrass pollen sensitive individual to pollen of such grasses or pollen of an immunologically related grass). Administration of such peptides may, for example, modify B-cell response to *Lol p* V allergen, T-cell response to *Lol p* V allergen or both responses. Isolated peptides can also be used to study the mechanism of immunotherapy of ryegrass pollen allergy and to design modified derivatives or analogues useful in immunotherapy.

The present invention also pertains to T cell clones which specifically recognize *Lol p* V peptides of the invention. These T cell clones may be suitable for isolation and molecular cloning of the gene for the T cell receptor which is specifically reactive with a peptide of the present invention. The T cell clones may be produced as described in Example 2, or as described in *Cellular Molecular Immunology*, Abul K. Abbas et al., W.B. Saunders Co. (1991) pg. 139. The present invention also pertains to soluble T cell receptors. These receptors may inhibit antigen-dependent activation of the relevant T cell subpopulation within an individual sensitive to *Lol p* V. Antibodies specifically reactive with such a T cell receptor can also be produced. Such antibodies may also be useful to block T-cell -MHC interaction in an individual. Methods for producing soluble T cell receptors are described in *Immunology; A Synthesis*, 2nd Ed., Edward S. Golub et al.,Sinaur Assoc, Sunderland Massachusetts. (1991) pp. 366-369.

It is also possible to modify the structure of a peptide of the invention for such purposes as increasing solubility, enhancing therapeutic or preventive efficacy, or stability (e.g., shelf life ex vivo, and resistance to proteolytic degradation in vivo). A modified peptide can be produced in which the amino acid sequence has been altered, such as by amino acid substitution, deletion, or addition, to modify immunogenicity and/or reduce allergenicity, or to which a component has been added for the same purpose.

For example, a peptide can be modified so that it maintains the ability to induce T cell anergy and bind MHC proteins without the ability to induce a strong proliferative response or possibly, any proliferative response when administered in immunogenic form. In this instance, critical binding residues for the T cell receptor can be determined using known techniques (e.g., substitution of each residue and determination of the presence or absence of T cell reactivity). Those residues shown to be essential to interact with the T cell receptor can be modified by replacing the essential amino acid with another, preferably similar amino acid residue (a conservative substitution) whose presence is shown to enhance, diminish but not eliminate, or not affect T cell reactivity. In addition, those amino acid residues which are not essential for T cell receptor interaction can be modified by being replaced by another amino acid whose incorporation may enhance, diminish or not affect T cell reactivity but does not eliminate binding to relevant MHC.

Additionally, peptides of the invention can be modified by replacing an amino acid shown to be essential to interact with the MHC protein complex with another, preferably similar amino acid residue (conservative substitution) whose presence is shown to enhance, diminish but not eliminate, or not affect T cell activity. In addition, amino acid residues which are not essential for interaction with the MHC protein complex but which still bind the MHC protein complex can be modified by being replaced by another amino acid whose incorporation may enhance, not affect, or diminish but not eliminate T cell reactivity. Preferred amino acid substitutions for non-essential amino acids include, but are not limited to substitutions with alanine, glutamic acid, or a methyl amino acid.

In order to enhance stability and/or reactivity, peptides of the invention can also be modified to incorporate one or more polymorphisms in the amino acid sequence of the protein allergen resulting from natural allelic variation. Additionally, D-amino acids, non-natural amino acids or non-amino acid analogues can be substituted or added to produce a modified peptide within the scope of this invention. Furthermore, peptides of the present invention can be modified using the polyethylene glycol (PEG) method of A. Sehon and co-workers (Wie et al. *supra*) to produce a protein or peptide conjugated with PEG. In addition, PEG can be added during chemical

synthesis of a protein or peptide of the invention. Modifications of peptides or portions thereof can also include reduction/ alyklation (Tarr in: *Methods of Protein Microcharacterization*, J.E. Silver ed. Humana Press, Clifton, NJ, pp 155-194 (1986)); acylation (Tarr, *supra*); chemical coupling to an appropriate carrier (Mishell and Shiigi, eds, *Selected Methods in Cellular Immunology*, WH Freeman, San Francisco, CA (1980); U.S. Patent 4,939,239; or mild formalin treatment (Marsh *International Archives of Allergy and Applied Immunology*, 41:199-215 (1971)).

To facilitate purification and potentially increase solubility of peptides of the invention, it is possible to add reporter group(s) to the peptide backbone. For example, poly-histidine can be added to a peptide to purify the peptide on immobilized metal ion affinity chromatography (Hochuli, E. et al., *Bio/Technology*, 6:1321-1325 (1988)). In addition, specific endoprotease cleavage sites can be introduced, if desired, between a reporter group and amino acid sequences of a peptide to facilitate isolation of peptides free of irrelevant sequences. In order to successfully desensitize an individual to a protein antigen, it may be necessary to increase the solubility of a peptide by adding functional groups to the peptide or by not including hydrophobic T cell epitopes or regions containing hydrophobic epitopes in the peptides or hydrophobic regions of the protein or peptide. It may also be necessary to replace hydrophobic residues with less hydrophobic residues, such changes effecting binding to a MHC complex or T cell receptor as described above.

To potentially aid proper antigen processing of T cell epitopes within a peptide, canonical protease sensitive sites can be recombinantly or synthetically engineered between regions, each comprising at least one T cell epitope. For example, charged amino acid pairs, such as KK or RR, can be introduced between regions within a peptide during construction of the peptide. The resulting peptide can be rendered sensitive to cathepsin and/or other trypsin-like enzymes cleavage to generate portions of the peptide containing one or more T cell epitopes. In addition, such charged amino acid residues can result in an increase in solubility of a peptide.

Site-directed mutagenesis of DNA encoding a peptide of the invention can be used to modify the structure of the peptide by methods known in the art. Such methods may, among others, include PCR with degenerate

oligonucleotides (Ho et al., *Gene*, 77:51-59 (1989)) or total synthesis of mutated genes (Hostomsky, Z. et al., *Biochem. Biophys. Res. Comm.*, 161:1056-1063 (1989)). To enhance bacterial expression, the aforementioned methods can be used in conjunction with other procedures to change the eucaryotic codons in DNA constructs encoding protein or peptides of the invention to ones preferentially used in *E. coli*, yeast, mammalian cells, or other eukaryotic cells.

Peptides or antibodies of the present invention can also be used for detecting and diagnosing ryegrass pollinosis. For example, this could be done by combining blood or blood products obtained from an individual to be assessed for sensitivity to ryegrass pollen with isolated peptides of *Lol p* V, under conditions appropriate for binding of components in the blood (e.g., antibodies, T-cells, B- cells) with the peptide(s) and determining the extent to which such binding occurs.

Isolated peptides of the invention when administered in a therapeutic regimen to a *Lol p* V-sensitive individual, or an individual allergic to an allergen cross-reactive with *Lol p* V, are capable of modifying the allergic response of the individual to *Lol p* V ryegrass pollen allergen or such cross-reactive allergen of the individual, and preferably are capable of modifying the B-cell response, T-cell response or both the B-cell and the T-cell response of the individual to the allergen. As used herein, modification of the allergic response of an individual sensitive to a ryegrass pollen allergen or cross-reactive allergen can be defined as non-responsiveness or diminution in symptoms to the allergen, as determined by standard clinical procedures (See e.g. Varney et al, *British Medical Journal*, 302:265-269 (1990)) including diminution in ryegrass pollen induced asthmatic symptoms. As referred to herein, a diminution in symptoms includes any reduction in allergic response of an individual to the allergen after the individual has completed a treatment regimen with a peptide or protein of the invention. This diminution may be subjective (i.e. the patient feels more comfortable in the presence of the allergen). Dimunition in symptoms can be determined clinically as well, using standard skin tests as is known in the art.

Lol p V peptides of the present invention which have T cell stimulating activity, and thus comprise at least one T cell epitope are particularly desirable

for therapeutic purposes. *Lol p* V peptides of the present invention which bind IgE and comprise at least one B cell epitope are particularly desirable for therapeutic purposes. In referring to an epitope, the epitope will be the basic element or smallest unit of recognition by a receptor, particularly immunoglobulins, histocompatibility antigens and T cell receptors where the epitope comprises amino acids essential to receptor recognition. Amino acid sequences which mimic those of the epitopes and which are capable of down regulating or reducing allergic response to *Lol p* V can also be used. T cell epitopes are believed to be involved in initiation and perpetuation of the immune response to a protein allergen which is responsible for the clinical symptoms of allergy. These T cell epitopes are thought to trigger early events at the level of the T helper cell by binding to an appropriate HLA molecule on the surface of an antigen presenting cell and stimulating the relevant T cell subpopulation. These events lead to T cell proliferation, lymphokine secretion, local inflammatory reactions, recruitment of additional immune cells to the site, and activation of the B cell cascade leading to production of antibodies. One isotype of these antibodies, IgE, is fundamentally important to the development of allergic symptoms and its production is influenced early in the cascade of events, at the level of the T helper cell, by the nature of the lymphokines secreted.

Exposure of ryegrass pollen allergic patients to isolated *Lol p* V peptides of the present invention which comprise at least one T cell epitope and are derived from *Lol p* V protein allergen may tolerize or anergize appropriate T cell subpopulations such that they become unresponsive to the protein allergen and do not participate in stimulating an immune response upon such exposure. In addition, administration of a peptide of the invention or portion thereof which comprises at least one T cell epitope may modify the lymphokine secretion profile as compared with exposure to the naturally-occurring *Lol p* V protein allergen or portion thereof (e.g. result in a decrease of IL-4 and/or an increase in IL-2). Furthermore, exposure to such peptide of the invention may influence T cell subpopulations which normally participate in the response to the naturally occurring allergen such that these T cells are drawn away from the site(s) of normal exposure to the allergen (e.g., nasal mucosa, skin, and lung) towards the site(s) of therapeutic administration of the

fragment or protein allergen. This redistribution of T cell subpopulations may ameliorate or reduce the ability of an individual's immune system to stimulate the usual immune response at the site of normal exposure to the allergen, resulting in a dimunution in allergic symptoms.

The isolated *Lol p* V peptides of the invention can be used in methods of diagnosing, treating and preventing allergic reactions to *Lol p* V allergen or a cross reactive protein allergen. Thus, the present invention provides diagnostic compositions for diagnosing allergy to Lol p V allergen or a cross-reactive protein allergen. Such compositions may comprise synthetically or recombinantly produced peptides derived from Lol p V and a pharmaceutically acceptable carrier or diluent. Such diagnostic compositions of the invention may be used in diagnostic tests for allergy such as radio-allergosorbent test (RAST), paper radioimmunosorbent test (PRIST), enzyme linked immunosorbent assay (ELISA), radioimmunoassay (RDA), immuno-radiometric assay (IRMA), luminesience immunoassay (LIA), histamine release assays and IgE immonoblots. The present invention also provides therapeutic compositions comprising isolated *Lol p* V peptides or portions thereof produced in a host cell transformed to express such *Lol p* V peptide or portion thereof and a pharmaceutically acceptable carrier or diluent. The therapeutic compositions of the invention may also comprise synthetically prepared *Lol p* V peptides and a pharmaceutically acceptable carrier or diluent. Administration of the therapeutic compositions of the present invention to an individual to be desensitized can be carried out using known techniques. *Lol p* V peptides or portions thereof may be administered to an individual in combination with, for example, an appropriate diluent, a carrier and/or an adjuvant. Pharmaceutically acceptable diluents include saline and aqueous buffer solutions. Pharmaceutically acceptable carriers include polyethylene glycol (Wie et al. (1981) *Int. Arch. Allergy Appl. Immunol.* 64:84-99) and liposomes (Strejan et al. (1984) *J. Neuroimmunol* 7: 27). For purposes of inducing T cell anergy, the therapeutic composition is preferably administered in nonimmunogenic form, e.g. it does not contain adjuvant. The therapeutic compositions of the invention are administered to ryegrass pollen-sensitive individuals or individuals sensitive to an allergen which is immunologically cross-reactive with ryegrass pollen allergen..

Administration of the therapeutic compositions of the present invention to an individual to be desensitized can be carried out using known procedures at dosages and for periods of time effective to reduce sensitivity (i.e., reduce the allergic response) of the individual to the allergen. Effective amounts of the therapeutic compositions will vary according to factors such as the degree of sensitivity of the individual to ryegrass pollen, the age, sex, and weight of the individual, and the ability of the protein or fragment thereof to elicit an antigenic response in the individual.

The active compound (i.e., protein or fragment thereof) may be administered in a convenient manner such as by injection (subcutaneous, intravenous, etc.), oral administration, inhalation, transdermal application, or rectal administration. Depending on the route of administration, the active compound may be coated within a material to protect the compound from the action of enzymes, acids and other natural conditions which may inactivate the compound.

For example, preferably about 1 µg- 3 mg and more preferably from about 20-500 µg of active compound (i.e., protein or fragment thereof) per dosage unit may be administered by injection. Dosage regimen may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily, weekly, monthly, quarterly, or yearly as needed or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

To administer a peptide by other than parenteral administration, it may be necessary to coat the protein with, or co-administer the protein with, a material to prevent its inactivation. For example, peptide or portion thereof may be co-administered with enzyme inhibitors or in liposomes. Enzyme inhibitors include pancreatic trypsin inhibitor, diisopropylfluorophosphate (DEP) and trasylol. Liposomes include water-in-oil-in-water CGF emulsions as well as conventional liposomes (Strejan *et al.*, (1984) J. Neuroimmunol. 7:27).

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethyline glycols, and mixtures thereof and in oils. Under ordinary

conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions of dispersion. In all cases, the composition must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glyceral, propylene glycol, and liquid polyetheylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thirmerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol and sorbitol or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about, including in the composition, an agent which delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating active compound (i.e., protein or peptide) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient (i.e., protein or peptide) plus any additional desired ingredient from a previously sterile-filtered solution thereof.

When a peptide of the invention is suitably protected, as described above, the peptide may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The peptide and other ingredients may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the individual's diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the composition and preparations may, of course, be varied and may conveniently be between about 5 to 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit contains between from about 10 µg to about 200 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum gragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservative, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the therapeutic compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit from as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

Various isolated peptides of the invention derived from ryegrass pollen protein *Lol p* V are shown in Fig. 2 and in Table 1. Peptides comprising at least two regions, each region comprising at least one T cell epitope of *Lol p* V are also within the scope of the invention. As used herein a region may include the amino acid sequence of a peptide of the invention as shown in Fig. 2 or the amino acid sequence of a portion of such peptide.

To obtain isolated peptides of the present invention, *Lol p* V is divided into non-overlapping peptides of desired length or overlapping peptides of desired lengths as discussed in Example 1 which can be produced recombinantly, or synthetically. Peptides comprising at least one B cell epitope are capable of binding IgE. Peptides comprising at least one T cell epitope are capable of eliciting a T cell response, such as T cell proliferation or lymphokine secretion, and/or are capable of inducing T cell anergy (i.e., tolerization). To determine peptides comprising at least one T cell epitope, isolated peptides are tested by, for example, T cell biology techniques, to

determine whether the peptides elicit a T cell response or induce T cell anergy. Those peptides found to elicit a T cell response or induce T cell anergy are defined as having T cell stimulating activity.

As discussed in Example 2, human T cell stimulating activity can be tested by culturing T cells obtained from an individual sensitive to *Lol p* V allergen, (i.e., an individual who has an IgE mediated immune response to *Lol p* V allergen) with a peptide derived from the allergen and determining whether proliferation of T cells occurs in response to the peptide as measured, e.g., by cellular uptake of tritiated thymidine. Stimulation indices for responses by T cells to peptides can be calculated as the maximum CPM in response to a peptide divided by the control CPM. A stimulation index (S.I.) equal to or greater than two times the background level is considered "positive". Positive results are used to calculate the stimulation index for each peptide for the patient tested. Preferred peptides of this invention comprise at least one T cell epitope and have a T cell stimulation index of greater than or equal to 2.0. A peptide having a T cell stimulation index of greater than or equal to 2.0 in a ryegrass pollen sensitive patient is considered useful as a therapeutic agent. Preferred peptides have a T cell stimulation index of at least 2.2, more preferably at least 2.5, more preferably at least 3.0, more preferably at least 3.4. For example, peptides of the invention having a T cell stimulation index of at least 2.5, as shown in Figs 4, 5, or 6, include peptides 14 (SEQ ID NO:16), 25 (SEQ ID NO:27), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 34 (SEQ ID NO:36).

In order to determine precise T cell epitopes by, for example, fine mapping techniques, a peptide having T cell stimulating activity and thus comprising at least one T cell epitope as determined by T cell biology techniques is modified by addition or deletion of amino acid residues at either the amino or carboxy terminus of the peptide and tested to determine a change in T cell reactivity to the modified peptide. Following this technique, peptides are selected and produced recombinantly or synthetically. Preferred therapeutic peptides are selected based on various factors, including the strength of the T cell response to the peptide (e.g., stimulation index), the frequency of the T cell response to the peptide in a population of individuals sensitive to ryegrass pollen, and the potential cross-reactivity of the peptide

with other allergens from other species of grasses as discussed earlier. The physical and chemical properties of these selected peptides (e.g., solubility, stability) are examined to determine whether the peptides are suitable for use in therapeutic compositions or whether the peptides require modification as described herein. The ability of the selected peptides or selected modified peptides to stimulate human T cells (e.g., induce proliferation, lymphokine secretion) is determined.

Additionally, preferred T cell epitope-containing therapeutic peptides of the invention do not bind immunoglobulin E (IgE) or bind IgE to a substantially lesser extent than the protein allergen from which the peptide is derived. The major complications of standard immunotherapy are IgE-mediated responses such as anaphylaxis. Immunoglobulin E is a mediator of anaphylactic reactions which result from the binding and cross-linking of antigen to IgE on mast cells or basophils and the release of mediators (e.g., histamine, serotonin, eosinophil chemotacic factors). Thus, anaphylaxis in a substantial percentage of a population of individuals sensitive to *Lol p* V could be avoided by the use in immunotherapy of a peptide or peptides which do not bind IgE in a substantial percentage (e.g., at least about 75%) of a population of individuals sensitive to *Lol p* V allergen, or if the peptide binds IgE, such binding does not result in the release of mediators from mast cells or basophils. The risk of anaphylaxis could be reduced by the use in immunotherapy of a peptide or peptides which have reduced IgE binding. Moreover, peptides which have minimal IgE stimulating activity are desirable for therapeutic effectiveness. Minimal IgE stimulating activity refers to IgE production that is less than the amount of IgE production and/or IL-4 production stimulated by the native *Lol p* V protein allergen.

A T cell epitope containing peptide of the invention, when administered to a ryegrass pollen-sensitive individual in a therapeutic treatment regimen is capable of modifying the allergic response of the individual to the allergen. Particularly, *Lol p* V peptides of the invention comprising at least one T cell epitope of *Lol p* V or at least two regions derived from *Lol p* V, each comprising at least one T cell epitope, when administered to an individual sensitive to ryegrass pollen are capable of modifying T cell response of the individual to the allergen.

An isolated *Lol p* V therapeutic peptide of the invention comprises at least one T cell epitope of the *Lol p* V and accordingly the peptide comprises at least approximately seven amino acid residues. For purposes of therapeutic effectiveness, preferred therapeutic compositions of the invention preferably comprise at least two T cell epitopes of *Lol p* V, and accordingly, the peptide comprises at least approximately eight amino acid residues and preferably at least fifteen amino acid residues. Additionally, therapeutic compositions comprising preferred isolated peptides of the invention preferably comprise a sufficient percentage of the T cell epitopes of the entire protein allergen such that a therapeutic regimen of administration of the composition to an individual sensitive to ryegrass pollen, results in T cells of the individual being tolerized to the protein allergen. Synthetically produced peptides of the invention comprising up to approximately forty-five amino acid residues in length, and most preferably up to approximately thirty amino acid residues in length are particularly desirable as increases in length may result in difficulty in peptide synthesis. Peptides of the invention may also be produced recombinantly as described above, and it is preferable that peptides of 45 amino acids or longer be produced recombinantly.

Peptides derived from the *Lol p* V protein allergen which can be used for therapeutic purposes comprise at least one T cell epitope of Lol p V and comprise all or a portion of the amino acid sequences of the following peptides: 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 12 (SEQ ID NO:14), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), 28 (SEQ ID NO:30), 33 (SEQ ID NO:35), and 34 (SEQ ID NO:36) (as shown in Fig. 2) preferrably, the peptide comprising an amino acid sequence of peptides: 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 12 (SEQ ID NO:14), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), 28 (SEQ ID NO:30), 33 (SEQ ID NO:35), and 34 (SEQ ID NO:36) (as shown in Fig. 2) has a mean T cell stimulation index equivalent to, or greater than the mean T cell stimulation index of the peptide from which it is derived as shown in Figs. 4, 5 or 6. Even more preferably peptides derived from the *Lol p* V protein allergen which can be used for therapeutic purposes comprise all or a portion of the amino acid sequences of the following peptides: 14 (SEQ ID NO:16), 19 (SEQ ID

NO:21). 22 (SEQ ID NO:24). 25 (SEQ ID NO:27). and 34 (SEQ ID NO:36) as shown in Fig. 2.

In one aspect of the present invention. a composition is provided comprising at least two peptides (e.g.. a physical mixture of at least two peptides). each comprising at least one T cell epitope of *Lol p V*. Such compositions can be administered in the form of a therapeutic composition with a pharmaceutically acceptable carrier or diluent. Additionally. therapeutically effective amounts of one or more of therapeutic compositions and comprising at least one peptide having a T cell epitope can be administered simultaneously or sequentially to an individual sensitive to ryegrass pollen.

Preferred compositions and preferred combinations of *Lol p* V peptides which can be administered simultaneously or sequentially (comprising peptides which comprise amino acid sequences shown in Fig. 2) include the following combinations:

14 (SEQ ID NO:16) and 22 (SEQ ID NO:24);

14 (SEQ ID NO:16) and 25 (SEQ ID NO:27);

19 (SEQ ID NO:21) and 22 (SEQ ID NO:24);

19 (SEQ ID NO:21) and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16). 22 (SEQ ID NO:24). and 25 (SEQ ID NO:27);

19 (SEQ ID NO:21). 22 (SEQ ID NO:24). and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16). 22 (SEQ ID NO:24). 25 (SEQ ID NO:27). and 34 (SEQ ID NO:36);

19 (SEQ ID NO:21). 22 (SEQ ID NO:24). 25 (SEQ ID NO:27). and 34 (SEQ ID NO:36);

14 (SEQ ID NO:16). 19 (SEQ ID NO:21). 22 (SEQ ID NO:24). and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16). 19 (SEQ ID NO:21). 22 (SEQ ID NO:24). 25 (SEQ ID NO:27). and 34 (SEQ ID NO:36);

6 (SEQ ID NO:8). 7 (SEQ ID NO:9). 9 (SEQ ID NO:11). 12 (SEQ ID NO:14). 14 (SEQ ID NO:16). 16 (SEQ ID NO:18). 19 (SEQ ID NO:21). 22 (SEQ ID NO:24). 25 (SEQ ID NO:27). 33 (SEQ ID NO:35). and 34 (SEQ ID NO:36);

6 (SEQ ID NO:8), 9 (SEQ ID NO:11), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 34 (SEQ ID NO:36); and

6 (SEQ ID NO:8), 9 (SEQ ID NO:11), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), and 34 (SEQ ID NO:36).

The present invention is further illustrated by the following non-limiting Figures and Examples:

EXAMPLES

EXAMPLE 1

***Lol p* V peptides**

The amino acid sequence of *Lol p* V (SEQ ID NO:2) was deduced from the cDNA sequence of clone 12R (SEQ ID NO:1) ATCC Number _____ as shown in Fig. 1. The cloning and sequencing of clone 12R encoding *Lol p* V (described as *Lol p* Ib.1) is described in PCT application publication number WO 93/04174. Thirty 12-mer and four 13-mer solid phase peptides were synthesized on a BT7400 manual peptide synthesising block (Biotech Instruments Ltd, UK). These peptides corresponded to the entire *Lol p* V sequence with four (or five) amino acid overlap. The peptides were synthesized on C-teminal Fmoc protected amino acid resins (25 mM auspep, Australia). The resins were washed and drained sequentially with 3 x 1 ml dimethylformamide (DMF: Auspep, Australia), and then treated with 1ml deprotecting solution (DP, 25% w/v piperidine in DMF; Suspep, Australia) for ten minutes to remove the Fmoc group from the first C-terminal amino acid. The wells were then washed and drained sequentially with 3 x 1 ml DMF, 2 x 1ml dichloromethane (DCM; Auspep, Australia) and finally 2 x 1 ml DMF. Ten fold exces of the selected amino acids were combined with activating reagent (BOP:HOBYT 1:1; Auspep, Australia), dissolved in 1 ml activating solution (Auspep, Australia) and added to the appropriate wells for coupling. The procedure was repeated until the desired peptide length was achieved.

The peptides were then treated with cleaving solution (90% v/v trifluoroacetic acid, 4% v/v phenol, 1% v/v ethanedithiol; Auspep, Australia) for six hours to facilitate peptide cleavage from the wells. The collected peptides were then precipitated in 50 ml cold diethylether (DEE: Auspep, Australia) and then isolated on Whatman's filter paper 1 (Whatman, UK). These filter papers were washed with 50 ml lyophilising solution (Auspep, Australia) and freeze dried. Peptides were subjected to analysis by HPLC and mass spectromety by Auspep Laboratories to check sequence and peptide purity.

TABLE 1

Peptide No.	Peptide Sequence N-terminus/C-terminus	Peptide MW*(D)	Peptide pI		IgE Binding Hydrophobicity
1	ADAGYTPAAAAT	1194	5.0	-	HYDROPHOBIC
2	AAATPATPAATPA	1111	6.1	-	HYDROPHOBIC
3	AATPAAAGGKAT	987	10.5	-	HYDROPHOBIC
4	GKATTDEQKLLE	1333	5.4	-	HYDROPHYLIC
5	KLLEDVNAGFKA	1306	8.0	+(3.0)	HYDROPHYLIC
6	GFKAAVAAAANA	1062	10.5	+(2.0)	HYDROPHOBIC
7	AANAPPADKFKI	1243	10.2	+(5.0)	HYDROPHYLIC
8	KFKIFEAAFSES	1405	7.3	-	HYDROPHOBIC
9	FSESSKGLLATS	1227	8.0	-	HYDROPHYLIC
10	LATSAAKAPGLI	1113	10.5	+(7.0)	HYDROPHYLIC
11	PGLIPKLNTAYD	1303	5.2	-	HYDROPHYLIC
12	TAYDVAYKAAEG	1259	5.3	-	HYDROPHOBIC
13	AAEGATPEAKYD	1223	4.5	+(4.0)	HYDROPHYLIC
14	AKYDAFVTALTE	1329	4.2	-	HYDROPHOBIC
15	ALTEGLRVIAGA	1171	8.2	-	HYDROPHOBIC
16	IAGALEVHAVKPA	1277	8.0	+(4.0)	HYDROPHOBIC
17	AVKPATEEVPAA	1183	4.4	-	HYDROPHYLIC
18	VPAAKIPTGELQI	1338	8.0	+(5.0)	HYDROPHYLIC
19	GELQIVDKIDAA	1272	4.4	+(11.0)	HYDROPHYLIC
20	IDAAFKIAATAA	1163	7.0	-	HYDROPHOBIC
21	ATAANAAPTNDK	1145	7.0	+(12.0)	HYDROPHOBIC
22	TNDKFTVFESAF	1407	4.2	-(2.0)	HYDROPHOBIC
23	ESAFNKALNECT	1327	4.4	+(10.0)	HYDROPHYLIC
24	NECTGGAYETYK	1336	6.1	+(2.8)	HYDROPHOBIC
25	ETYKFIPSLEAA	1370	4.4	+(3.0)	HYDROPHOBIC
26	LEAAVKQAYAAT	1236	7.1	-	HYDROPHYLIC
27	YAATVAAPEVKY	1283	7.1	+(2.0)	HYDROPHOBIC
28	EVKYAVFEAALT	1342	4.4	-	HYDROPHOBIC
29	AALTKAITAMTQA	1292	10.4	+(2.5)	HYDROPHOBIC

30	AMTQAQKAGKPA	1202	10.9	+(4.7)	HYROPOHYLIC
31	GKPAAAAATAAA	971	10.5	-	HYDROPHOBIC
32	TAAATVATAAAT	1020	6.1	-	HYDROPHOBIC
33	AAATAAAGAATA	918	8.1	-	HYDROPHOBIC
34	GAATAAAGGYKA	1009	10.3	+(12.0)	HYDROPHOBIC

Peptide Dot Blot Immunoassays

The peptides were dissolved in water to give a 1.6 mM stock solution. Insoluble peptides were dissolved by sonication (Branson Sofifier 450, USA) for one minute.

The peptides were immobilized onto nitrocellulose (NC) filter by using a modified method published elsewhere (Sithigonglu (1991) J. Immunol. Methods, 141:23-32). Two µl of the peptide stock solutions were spotted on the NC filter (Schleicher & Schuell, W. Germany) about 1 cm apart. After the strips were dried and baked at 80°C for one hour, they were fixed by exposure, in a tightly sealed plastic box, to vapor from 0.2% glutaraldehyde in PBS at room temperature (18°C) for one hour. After washing thoroughly with distilled water, the NC filters were blocked with Blotto (10% non-fat dry milk in PBS) for two hours and washed in 1X Tween-PBS (0.1% Tween-20 in PBS) and 2X PBS, five minutes each.

The peptides were then screened for human IgE and mouse/rabbit IgG epitopes with individual sera (diluted 1:4 in PBS with 0.5% BSA), MAbs FMC A7 (Smart et al. (1983) Int. Arch. Allergy Clin. Immunol. 72:243) and polyclonal rabbit anti-Lol p V (Lol p Ib) antibody.

Our of the fifty highly ryegrass pollen allergic subjects, twenty-three had IgE that bound to at least one peptide. Although the majority of the subjects showed mild to moderate IgE-reactivity, there were several showing high reactivity. The dot blot analysis for IgE binding is shown in Fig. 7.

The intensity of each dot blot was measured by densitometer (Pharmacia LKB Ultra Scan XL, Sweden). Values are given in arbitrary densitometric units and shown in Fig. 8. Densitometric values greater than two were considered as positive binding in comparison to the background. For every positive binding in comparison to the background. For every peptide, the sera and antibody values have been added (score/peptide) and divided by the number of positive sera to express the final value as an average (score/sera). Sera C1 and C2 were not grass allergic and were used as negative controls. C refers to crude ryegrass pollen extract (1µg/dot) as positive control (see example 3).

Sera 1, 14, 36, 42 and 48 indicated substantial affinity towards a selection of specific peptides (e.g. peptides 19 (SEQ ID NO:21), 34 (SEQ ID

NO:36), and 7 (SEQ ID NO:9)), which were classified as immunodominant linear allergenic B-cell epitopes of Lol p V (Figures 7 and 8).

Peptide numbers 7 (SEQ ID NO:9), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 21 (SEQ ID NO:23), 23 (SEQ ID NO:25), 30 (SEQ ID NO:32) and 34 (SEQ ID NO:36) indicated the highest human IgE-reactivity and were thus classified as major linear allergenic B-cell epitopes of Lol p V. MAb FMC-A7, and rabbit polyclonal anti-Lol p Ib antiserum were analyzed for IgG binding to the peptides. This data is shown in Fig. 7. The MAbs and rabbit antiserum generally showed low binding to the peptides. Peptide numbers 1 (SEQ ID NO:3), 7 (SEQ ID NO:9), 19 (SEQ ID NO:21), 23 (SEQ ID NO:25), 24 (SEQ ID NO:26) and 28 (SEQ ID NO:30) indicated the highest mouse/rabbit IgG-reactivity.

EXAMPLE 2

1. MATERIALS AND METHODS

Crude Rye and Bermuda Grass Pollen Extracts

Rye grass (Lolium perenne) and Bermuda grass (Cynodon dactylon) pollen was obtained from Greer Laboratories (USA) as dry non-defatted extracts. Pollen allergens were extracted in 0.001M NH_4HCO_3 (10% w/v), centrifuged and the supernatants dialysed against Phosphate buffered saline (PBS) overnight. The supernatants were then passed through a 0.20 µm filter and protein concentration was determined using the BioRad Microassay (Bio-Rad, USA) according to the manufacturer's instructions.

Lol p V

Lol p V was purified from crude rye grass pollen extracts by fluid phase liquid chromatography (FPLC). Briefly, the crude rye grass pollen extract was subjected to a buffer change from PBS to 20 mM Tris pH12 (buffer A) on an Econo-Pac 10DG column (Bio-Rad, USA). This extract, 5 mg, was injected onto an ion-exchange FPLC column (Mono Q HR 5/5; Pharmacia, Sweden), pre-equilibrated sequentially with buffer A for five minutes, then buffer B (20mM Tris, pH 7.0) for ten minutes and finally with

buffer A for a further five minutes. The column was washed with buffer A for ten minutes to allow for the binding of Lol p V as well as for the elution of other proteins with pI values equal to or higher than twelve. Elution of Lol p V was performed with buffer B at a constant flow rate of 1ml/minute for fifteen minutes at room temperature. Fractions of 1ml were collected every minute and then analyzed by SDS-PAGE and immunoblotting. Fractions containing Lol p V were pooled and the protein concentration was determined.

***Lol p* V peptides**

The same Lol p V peptides were produced as described in Example 1. Peptide 13 (SEQ ID NO:15) was produced in very small amounts only and could not be tested in this study.

Generation of allergen-reactive T cell lines and clones

Peripheral blood mononuclear cells (PBMC) from a healthy adult with a positive skin test to rye grass pollen, were isolated from heparinised blood samples by centrifugation on a Ficoll-Paque (Pharmacia, Sweden) density gradient. Initially 1 x 10^6/ml PBMC were stimulated with 5 µg/ml of Lol p V in a 96-well U-bottomed microtiter plates (Linbro, USA). All cells were cultured in RPMI-1640 (ICN, Australia) tissue culture medium supplemented with 1% w/v L-glutamine (ICN, Australia), 0.125% w/v gentomicin (Delta West, Australia) and 5% v/v heat-inactivated human A+ serum. Cultures were performed at 37°C in 5% v/v CO_2 atmosphere.

Seven days after initiation of culture, ten units/ml of human recombinant IL-2 (rIL-2; Cetus, USA) was added to the wells. On day 14 the T cell line was expanded by transferring the cells to a 24-well tissue culture plate (Costar, USA) and adding 5 x 10^5/ml irradiated (2000 rads) autologous PBMC, 2.5 µg/ml Lol p V, 10-20 units/ml rIL-2, 5-10 units/ml Lymphocult (Biotest, Germany) and 0.1µg/ml PHA (Wellcome, UK). The line was restimulated every 8-10 days as described.

T cells were then cloned by limiting dilution of the line. For this, T cells were seeded at 300, 30, 3, 1 and 0.3 cells/well in 96-well U-bottomed microtiter plates together with 1x10^6/ml irradiated PBMC, 2.5 µg/ml Lol p

V, 20 units/ml rIL-2, 10 units/ml lymphocult and 0.1 µg/ml PHA. The clones were maintained with rIL-2 every four days and irradiated autologous PBMC, Lol p V, rIL-2 and Lymphocult were added every 8-10 days.

Before use in proliferation assays the lines and clones were rested for 7-8 days after the last addition of feeder cells, allergen and IL-2.

Proliferation assays

$5x10^5$/ml T cells were cultured with $5x10^5$/ml irradiated autologous PBMC and Lol p V (0.019-2.5 µg/ml or 0.625-20µg/ml) or Lol p V peptides (1 and 10µg/ml) in 96-well U-bottomed microtiter plates for 88 hours, then pulsed with 1µCi/well tritiated methyl thymidine ([^{3}H]TdR; Amersham, USA) for eight hours prior to harvesting onto glass-fibre filters. Control cultures included cells with no allergen, cells with PHA, T cells alone and irradiated PBMC cells alone. The counts per minute (CPM) for each filter was determined using a Liquid Scintillation counter (LKB Wallace, Finland).

Immunophenotyping

T cells were stained by direct or indirect immunofluorescence using the following monoclonal antibodies: CD2, CD3,CD4, CD5, CD8, CD11b, CD16, CD19, CD25, CD45RO, CD45RA, MHC class II TcRαβ TcRγδ, k light chain and λ light chain. For indirect immunofluorescence, FITC-conjugated sheep-anti-mouse Ig $F(ab)_2$ (Silenus, Australia) was used. Cells were then analyzed using a FACScan flow cytometer (Becton-Dickinson, USA).

2. RESULTS

Allergen reactivity of the T cell line

On day 34 of culture, the T cell line was tested for reactivity to rye and Bermuda grass pollen allergens. Over a range of concentrations there was a strong proliferative response to the inducing allergen Lol p V and there was a lower response to crude rye grass pollen extract (Fig. 3). There was no response to the crude Bermuda grass pollen extract.

Reactivity of the T cell line and clones to Lol p V synthetic peptides

The T cell line was first tested for reactivity to the Lol p V synthetic peptides on day 14, but no significant response was detected. However, at day 27, a strong response to peptide 14 (SEQ ID NO:16) was observed. By day 49, the strong reactivity to peptide 14 (SEQ ID NO:16) was maintained but there was additional weaker response to peptide 25 (SEQ ID NO:27) (Fig. 4), consistent with the polyclonal nature of the T cell line. The amino acid sequence of peptides 14 (SEQ ID NO:16) and 25 (SEQ ID NO:27) are shown in Table 2.

T cell clones were generated from the T cell line at day 17. One of the eight T cell clones obtained, designated A12, showed a strong response to peptide 14 (SEQ ID NO:16) only (Fig. 5). The stimulation index of the clone A12 response to peptide 14 (SEQ ID NO:16) was 137, compared with 4.8 for the T cell line at day 49, correlating with selection of peptide 14-specific T cells.

Phenotypic analysis of the T cell line

The phenotype of the T cell line at day 47 was analyzed by flow cytometry using a panel of monoclonal antibodies against cell surface markers. The line expressed CD2, CD3, CD5, CD4, CD25, CD45RO, MHC class II and TcRαβ, consistent with the phenotype of activated mature helper T cells.

Table 2

Amino acid sequences of the T cell reactive Lol p IX peptides

Peptide	Amino Acid Sequence
14 (SEQ ID NO:16)	AKYDAFVTALTE
25 (SEQ ID NO:27)	ETYKFIPSLEAA

EXAMPLE 3

1. MATERIALS AND METHODS

Another T cell line was produced from the same patient described in Example 2 in accordance with the procedure discussed in Example 2. Proliferation assays were conducted using the *Lol p* V synthetic peptides described in Example 1 in accordance with the procedure discussed in Example 2.

2. RESULTS

The T cell line was tested for reactivity to the *Lol p* V synthetic peptides The results are shown in Fig. 6. Proliferation is given with background proliferation subtracted. Background proliferation (proliferation to medium alone) was 2500 cpm. Stimulation indexes were calculated from the results shown by adding 2500 cpm to the values shown in Fig. 6 and dividing by 2500. Peptides 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 9 (SEQ ID NO:11), 12 (SEQ ID NO:14), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 28 (SEQ ID NO:30), 33 (SEQ ID NO:35), and 34 (SEQ ID NO:36) had stimulation indexes of greater than or equal to 2.0 as shown in Fig. 6. The most reactive peptides are 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 34 (SEQ ID NO:36), with respective T cell stimulation indexes of 2.9, 3.4 and 2.5.

EXAMPLE 4

IgE BINDING STUDIES WITH PEPTIDE 34 USING INHIBITION PEPTIDE DOT BLOTS AND INHIBITION ELISA

A. Inhibition peptide dot-blots.

Brief methodology:

Peptides 34 (SEQ ID NO:36), 6 (SEQ ID NO:8), 24 (SEQ ID NO:26) and 31 (SEQ ID NO:33) (last three peptides used as negative controls) as well as FPLC purified *Lol p* V and crude ryegrass pollen extract (designated as IX

and C. respectively, in Fig. 9 and used as positive controls) were immobilized on NC, baked and gluteraldehyde fixed as previously described. Two separate lots of serum (from subject Girgis possessing peptide 34-specific IgE antibodies; diluted 1:4) were preincubated overnight at RT (18° C) either with 50 µg crude ryegrass pollen extract per ml diluted serum (designated as inhibited serum on the figure) or without the crude extract (designated as control serum). Peptide blots were then separately incubated in the control and the crude extract preincubated serum overnight followed by ^{125}I labelled anti-human IgE antibodies and exposed to X-ray film for up to 96 hrs.

Results:

The control serum gave strong IgE-binding to peptide 34 (SEQ ID NO:36) and the positive controls *Lol p* IX and crude ryegrass pollen extract. The other control peptides (e.g. alanine-rich peptide 31 (SEQ ID NO:33), and peptides 6 (SEQ ID NO:8), 24 (SEQ ID NO:26)) gave no IgE-binding.

The serum preincubated with crude ryegrass pollen extract gave a reduced IgE-binding to peptide 34 (SEQ ID NO:36) as well as to the positive controls *Lol p* V and the crude ryegrass pollen extract.

Conclusions:

These results demonstrate that this individual has peptide 34-specific IgE antibodies which can be inhibited by crude ryegrass pollen extract.

B. Inhibition ELISA.

Brief methodology:

Peptides 34 (SEQ ID NO:36), 6 (SEQ ID NO:8), 24 (SEQ ID NO:26) and 31 (SEQ ID NO:33) (last three peptides used as negative controls) and crude ryegrass pollen extract (used as positive control) were coated on microtiter plates. Serum of the individual (diluted 1:4) was preincubated either with or without different concentrations of crude ryegrass pollen extract overnight at RT. The peptide/crude extract coated wells were then incubated with the control and crude extract preincubated serum at the different crude extract concentrations. Plates were washed and incubated with anti-human

IgE raised in rabbit followed by HRP-conjugated anti-rabbit antibodies. The plates were devleoped with the peroxidase substrate 1. 2-Phenylenediamine and the colour reaction was read at 492nm.

Results:

As shown in Fig. 10. the control serum gave IgE-binding to peptide 34 (SEQ ID NO:36) and crude ryegrass pollen extract. The gradual increase in the concentration of crude extract gave a gradual decrease in IgE-binding to both peptide 34 (SEQ ID NO:36) and crude extract. The control peptides all displayed a significantly lower IgE-binding and there was no significant correlation between the gradual decrease in IgE-binding with increasing inhibitor concentration. The binding shown by the control peptides is due to background. that is non-specific IgE-binding to the peptides or the well due to assay conditions. However, even though a slight background was observed with ELISA. the IgE-binding to peptide 34 (SEQ ID NO:36) and crude extract was higher than the control peptides.

Conclusions:

As with the inhibition dot-blot results. the inhibition ELISA results indicates significant IgE-binding to peptide 34 (SEQ ID NO:36) in this individual.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention also includes all such variations and modifications. The invention also includes all of the steps. features. compositions and compounds referred to or indicated in this specification. individually or collectively. and any and all combinations of any two or more of said steps or features.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: UNIVERSITY OF MELBOURNE
(B) STREET: Grattan Street
(C) CITY: Parkville
(D) STATE: Victoria
(E) COUNTRY: AUSTRALIA
(F) POSTAL CODE (ZIP): 3052
(G) TELEPHONE: 3-613-344-4000
(H) TELEFAX: 3-613-344-7628

(ii) TITLE OF INVENTION: T CELL EPITOPES OF RYEGRASS POLLEN ALLERGEN

(iii) NUMBER OF SEQUENCES: 36

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: ASCII Text

(v) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US07/866,679
(B) FILING DATE: 09-APR-1992

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: John M. Slattery
Davies Collison Cave
1 Little Collins Street, Melbourne 3000
AUSTRALIA
(B) REGISTRATION NUMBER:
(C) REFERENCE/DOCKET NUMBER: IPC-011C3PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 613-254-2777
(B) TELEFAX: 613-254-2770

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 42..942

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 115..942

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CGCTATCCCT CCCTCGTACA AACAAACGCA AGAGCAGCA ATG GCC GTC CAG AAG          54
                                           Met Ala Val Gln Lys
                                           -25

TAC ACG GTG GCT CTA TTC CTC GCC GTG GCC CTC GTG GCG GGC CCG GCC        102
Tyr Thr Val Ala Leu Phe Leu Ala Val Ala Leu Val Ala Gly Pro Ala
-20                 -15                 -10                 -5

GCC TCC TAC GCC GCT GAC GCC GGC TAC ACC CCC GCA GCC GCG GCC ACC        150
Ala Ser Tyr Ala Ala Asp Ala Gly Tyr Thr Pro Ala Ala Ala Ala Thr
                1                   5                  10

CCG GCT ACT CCT GCT GCC ACC CCG GCT GCG GCT GGA GGG AAG GCG ACG        198
Pro Ala Thr Pro Ala Ala Thr Pro Ala Ala Ala Gly Gly Lys Ala Thr
        15                  20                  25

ACC GAC GAG CAG AAG CTG CTG GAG GAC GTC AAC GCT GGC TTC AAG GCA        246
Thr Asp Glu Gln Lys Leu Leu Glu Asp Val Asn Ala Gly Phe Lys Ala
    30                  35                  40

GCC GTG GCC GCC GCT GCC AAC GCC CCT CCG GCG GAC AAG TTC AAG ATC        294
Ala Val Ala Ala Ala Ala Asn Ala Pro Pro Ala Asp Lys Phe Lys Ile
45                  50                  55                  60

TTC GAG GCC GCC TTC TCC GAG TCC TCC AAG GGC CTC CTC GCC ACC TCC        342
Phe Glu Ala Ala Phe Ser Glu Ser Ser Lys Gly Leu Leu Ala Thr Ser
                65                  70                  75

GCC GCC AAG GCA CCC GGC CTC ATC CCC AAG CTC GAC ACC GCC TAC GAC        390
Ala Ala Lys Ala Pro Gly Leu Ile Pro Lys Leu Asp Thr Ala Tyr Asp
            80                  85                  90

GTC GCC TAC AAG GCC GCC GAG GGC GCC ACC CCC GAG GCC AAG TAC GAC        438
Val Ala Tyr Lys Ala Ala Glu Gly Ala Thr Pro Glu Ala Lys Tyr Asp
        95                  100                 105

GCC TTC GTC ACT GCC CTC ACC GAA GCG CTC CGC GTC ATC GCC GGC GCC        486
Ala Phe Val Thr Ala Leu Thr Glu Ala Leu Arg Val Ile Ala Gly Ala
    110                 115                 120

CTC GAG GTC CAC GCC GTC AAG CCC GCC ACC GAG GAG GTC CCT GCT GCT        534
Leu Glu Val His Ala Val Lys Pro Ala Thr Glu Glu Val Pro Ala Ala
125                 130                 135                 140

AAG ATC CCC ACC GGT GAG CTG CAG ATC GTT GAC AAG ATC GAT GCT GCC        582
Lys Ile Pro Thr Gly Glu Leu Gln Ile Val Asp Lys Ile Asp Ala Ala
                145                 150                 155
```

```
TTC AAG ATC GCA GCC ACC GCC GCC AAC GCC GCC CCC ACC AAC GAT AAG          630
Phe Lys Ile Ala Ala Thr Ala Ala Asn Ala Ala Pro Thr Asn Asp Lys
            160                 165                 170

TTC ACC GTC TTC GAG AGT GCC TTC AAC AAG GCC CTC AAT GAG TGC ACG          678
Phe Thr Val Phe Glu Ser Ala Phe Asn Lys Ala Leu Asn Glu Cys Thr
        175                 180                 185

GGC GGC GCC TAT GAG ACC TAC AAG TTC ATC CCC TCC CTC GAG GCC GCG          726
Gly Gly Ala Tyr Glu Thr Tyr Lys Phe Ile Pro Ser Leu Glu Ala Ala
    190                 195                 200

GTC AAG CAG GCC TAC GCC GCC ACC GTC GCC GCC GCG CCC GAG GTC AAG          774
Val Lys Gln Ala Tyr Ala Ala Thr Val Ala Ala Ala Pro Glu Val Lys
205                 210                 215                 220

TAC GCC GTC TTT GAG GCC GCG CTG ACC AAG GCC ATC ACC GCC ATG ACC          822
Tyr Ala Val Phe Glu Ala Ala Leu Thr Lys Ala Ile Thr Ala Met Thr
                225                 230                 235

CAG GCA CAG AAG GCC GGC AAA CCC GCT GCC GCC GCT GCC ACA GGC GCC          870
Gln Ala Gln Lys Ala Gly Lys Pro Ala Ala Ala Ala Ala Thr Gly Ala
        240                 245                 250

GCA ACC GTT GCC ACC GGC GCC GCA ACC GCC GCC GCC GGT GCT GCC ACC          918
Ala Thr Val Ala Thr Gly Ala Ala Thr Ala Ala Ala Gly Ala Ala Thr
    255                 260                 265

GCC GCT GCT GGT GGC TAC AAA GCC TGATCAGCTT GCTAATATAC TACTGAACGT         972
Ala Ala Ala Gly Gly Tyr Lys Ala
270                 275

ATGTATGTGC ATGATCCGGG CGGCGAGTCG TTTTGTTGAT AATTAATCTT CGTTTTCGTT        1032

TCATGCAGCC GCGATCGAGA GGGCTTGCAT GCTTGTAATA ATTCAATATT TTTCATTTCT        1092

TTTTGAATCT GTAAATCCCC ATGACAAGTA GTGGGATCAA GTCGGCATGT ATCACCGTTG        1152

ATGCGAGTTT AACGATGGGG AGTTTATCAA AGAATTTATT ATTAAAAAAA AAAAAAAAAA        1212

AAAAAAAAAA AAAAAAAGAG CACGCTATCC CTCCCTCGTA CAAACAAACG CAAGAGCAGC        1272
```

AATGGCCGTC CAGAAGTACA CGGTGGCTCT ATTCCTCGCC GTGGCCCTCG TGGCGGGCCC 1332

GGCCGCCTCC TACGCCGCTG ACGCTTAAAC ATTCATATCA TCAT 1376

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 301 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Val Gln Lys Tyr Thr Val Ala Leu Phe Leu Ala Val Ala Leu
-25 -20 -15 -10

Val Ala Gly Pro Ala Ala Ser Tyr Ala Ala Asp Ala Gly Tyr Thr Pro
-5 1 5

Ala Ala Ala Ala Thr Pro Ala Thr Pro Ala Ala Thr Pro Ala Ala Ala
10 15 20

Gly Gly Lys Ala Thr Thr Asp Glu Gln Lys Leu Leu Glu Asp Val Asn
25 30 35

Ala Gly Phe Lys Ala Ala Val Ala Ala Ala Ala Asn Ala Pro Pro Ala
40 45 50 55

Asp Lys Phe Lys Ile Phe Glu Ala Ala Phe Ser Glu Ser Ser Lys Gly
60 65 70

Leu Leu Ala Thr Ser Ala Ala Lys Ala Pro Gly Leu Ile Pro Lys Leu
75 80 85

Asp Thr Ala Tyr Asp Val Ala Tyr Lys Ala Ala Glu Gly Ala Thr Pro
90 95 100

Glu Ala Lys Tyr Asp Ala Phe Val Thr Ala Leu Thr Glu Ala Leu Arg
105 110 115

Val Ile Ala Gly Ala Leu Glu Val His Ala Val Lys Pro Ala Thr Glu
120 125 130 135

Glu Val Pro Ala Ala Lys Ile Pro Thr Gly Glu Leu Gln Ile Val Asp
140 145 150

Lys Ile Asp Ala Ala Phe Lys Ile Ala Ala Thr Ala Ala Asn Ala Ala
155 160 165

Pro Thr Asn Asp Lys Phe Thr Val Phe Glu Ser Ala Phe Asn Lys Ala
170 175 180

Leu Asn Glu Cys Thr Gly Gly Ala Tyr Glu Thr Tyr Lys Phe Ile Pro
185 190 195

Ser Leu Glu Ala Ala Val Lys Gln Ala Tyr Ala Ala Thr Val Ala Ala
200 205 210 215

Ala Pro Glu Val Lys Tyr Ala Val Phe Glu Ala Ala Leu Thr Lys Ala

220 225 230

Ile Thr Ala Met Thr Gln Ala Gln Lys Ala Gly Lys Pro Ala Ala Ala
235 240 245

Ala Ala Thr Gly Ala Ala Thr Val Ala Thr Gly Ala Ala Thr Ala Ala
250 255 260

Ala Gly Ala Ala Thr Ala Ala Ala Gly Gly Tyr Lys Ala
265 270 275

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ala Asp Ala Gly Tyr Thr Pro Ala Ala Ala Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Ala Ala Thr Pro Ala Thr Pro Ala Ala Thr Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Ala Thr Pro Ala Ala Ala Gly Gly Lys Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gly Lys Ala Thr Thr Asp Glu Gln Lys Leu Leu Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Lys Leu Leu Glu Asp Val Asn Ala Gly Phe Lys Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Gly Phe Lys Ala Ala Val Ala Ala Ala Ala Asn Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ala Ala Asn Ala Pro Pro Ala Asp Lys Phe Lys Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Lys Phe Lys Ile Phe Glu Ala Ala Phe Ser Glu Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Phe Ser Glu Ser Ser Lys Gly Leu Leu Ala Thr Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ala Thr Ser Ala Ala Lys Ala Pro Gly Leu Ile

1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Pro Gly Leu Ile Pro Lys Leu Asn Thr Ala Tyr Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Ala Tyr Asp Val Ala Tyr Lys Ala Ala Glu Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Ala Glu Gly Ala Thr Pro Glu Ala Lys Tyr Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Lys Tyr Asp Ala Phe Val Thr Ala Leu Thr Glu
1 5 10

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Leu Thr Glu Gly Leu Arg Val Ile Ala Gly Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ile Ala Gly Ala Leu Glu Val His Ala Val Lys Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala Val Lys Pro Ala Thr Glu Glu Val Pro Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Val Pro Ala Ala Lys Ile Pro Thr Gly Glu Leu Gln Ile
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Gly Glu Leu Gln Ile Val Asp Lys Ile Asp Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Ile Asp Ala Ala Phe Lys Ile Ala Ala Thr Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Ala Thr Ala Ala Asn Ala Ala Pro Thr Asn Asp Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Thr Asn Asp Lys Phe Thr Val Phe Glu Ser Ala Phe
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Glu Ala Ser Phe Asn Lys Ala Leu Asn Glu Cys Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Asn Glu Cys Thr Gly Gly Ala Tyr Glu Thr Tyr Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Glu Thr Tyr Lys Phe Ile Pro Ser Leu Glu Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Leu Glu Ala Ala Val Lys Gln Ala Tyr Ala Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Tyr Ala Ala Thr Val Ala Ala Pro Glu Val Lys Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Glu Val Lys Tyr Ala Val Phe Glu Ala Ala Leu Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Ala Ala Leu Thr Lys Ala Ile Thr Ala Met Thr Gln Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Met Thr Gln Ala Gln Lys Ala Gly Lys Pro Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Lys Pro Ala Ala Ala Ala Ala Thr Ala Ala Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Thr Ala Ala Ala Thr Val Ala Thr Ala Ala Ala Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Ala Ala Ala Thr Ala Ala Ala Gly Ala Ala Thr Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Gly Ala Ala Thr Ala Ala Ala Gly Gly Tyr Lys Ala
1 5 10

Claims

What is claimed is:

1. An isolated peptide of *Lol p* V or a homologue thereof wherein said peptide comprises an amino acid sequence selected from the group consisting of amino acid sequences as shown in Fig. 2 of peptides 1 (SEQ ID NO:3), 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 21 (SEQ ID NO:23), 22 (SEQ ID NO:24), 23 (SEQ ID NO:25), 24 (SEQ ID NO:26), 25 (SEQ ID NO:27), 28 (SEQ ID NO:30), 30 (SEQ ID NO:32), 33 (SEQ ID NO:35) and 34 (SEQ ID NO:36).

2. An isolated peptide of claim 1 wherein said peptide comprises at least one T cell epitope of Lol p V, said peptide comprising an amino acid sequence selected from the group consisting of the amino acid sequences as shown in Fig. 2 of peptides 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 12 (SEQ ID NO:14), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), 28 (SEQ ID NO:30), 33 (SEQ ID NO:35) and 34 (SEQ ID NO:36).

3. An isolated peptide of claim 1 wherein, said peptide comprising at least one B cell epitope of Lol p V, said peptide comprising an amino acid sequence selected from the group consisting of amino acid sequences as shown in Fig. 2 of peptides 1 (SEQ ID NO:3), 7 (SEQ ID NO:9), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 21 (SEQID NO:23), 23 (SEQ ID NO:25), 24 (SEQ ID NO:26), 28 (SEQ ID NO:30), 30 (SEQ ID NO:32) and 34 (SEQ ID NO:36).

4. A portion of an isolated peptide of claim 2 which has a T cell stimulation index of at least 2.0.

5. A portion of an isolated peptide of claim 2 which has a T cell stimulation index approximately equivalent to or greater than the T cell stimulation index of said peptide as shown in Figs. 4 or 6.

6. An isolated peptide of claim 2 which, when administered to an individual sensitive to *Lol p* V allergen, induces T cell anergy in the individual or modifies the lymphokine secretion profile of T cells in the individual.

7. An isolated peptide of claim 2 which does not bind immunoglobulin E specific for *Lol p* V in a substantial percentage of individuals sensitive to *Lol p* V, or if binding of the peptide or portion thereof to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of individuals sensitive to *Lol p* V.

8. An isolated peptide of claim 2 which binds immunoglobulin E to a substantially lesser extent than purified native *Lol p* V binds immunoglobulin E.

9. An isolated nucleic acid sequence having a sequence encoding a peptide of claim 1.

10. A functional equivalent of a nucleic acid sequence encoding a peptide of claim 1.

11. An isolated peptide which is immunologically cross-reactive with T cells reactive with a peptide of claim 2.

12. An isolated peptide of Lol p V or a homologue thereof wherein said peptide has a T cell stimulation index of at least about 2.2.

13. An isolated peptide of claim 12 wherein said T cell stimulation index is at least about 3.0.

14. A modified peptide of claim 1.

15. A modified peptide of claim 2.

16. A modified peptide of claim 15 which does not bind immunoglobulin E specific for *Lol p V* in a substantial percentage of individuals sensitive to *Lol p V*, or if binding of the peptide to said immunoglobulin E occurs, such binding does not result in release of mediators from mast cells or basophils in a substantial percentage of individuals sensitive to *Lol p V*.

17. A modified peptide of claim 14 which modifies, in an individual sensitive to *Lol p* V to whom it is administered, the allergic response of the individual to a *Lol p* V allergen.

18. A monoclonal antibody specifically reactive with a peptide of claim 1.

19. An isolated peptide produced in a host cell transformed with the nucleic acid of claim 9.

20. An isolated peptide produced in a host cell transformed with the nucleic acid of claim 10.

21. An expression vector comprising a nucleic acid sequence coding for a peptide of claim 1.

22. An expression vector comprising the functional equivalent of a sequence coding for a peptide of claim 1.

23. A therapeutic composition comprising at least one isolated peptide of claim 2 and a pharmaceutically acceptable carrier or diluent.

24. A therapeutic composition comprising at least one isolated peptide of claim 15 and a pharmaceutically acceptable carrier or diluent.

25. The use of a composition according to claim 23 or 24 for the manufacture of a medicament for treating sensitivity to *Lol p* V allergen or an allergen which is immunologically cross reactive with *Lol p* V allergen in an individual.

26. A method for treating sensitivity to *Lol p* V allergen or an allergen which is immunologically cross reactive with *Lol p* V allergen in an individual comprising administering to said individual a therapeutically effective amount of a composition of claim 23 or 24.

27. A method of detecting sensitivity to *Lol p* V in an individual, comprising combining a blood sample obtained from the individual with at least one peptide of claim 1, under conditions appropriate for binding of blood components with the peptide, and determining the extent to which such binding occurs as indicative of sensitivity in the individual to ryegrass pollen.

28. A method of claim 27 wherein the extent to which binding occurs is determined by assessing B cell function, T cell function, T cell proliferation or a combination of T cell function and B cell proliferation.

29. A use of at least two compositions according to claim 23 or 24 for the manufacture of a medicament for treating sensitivity to *Lol p* V allergen which is immunologically cross reactive with ryegrass pollen allergen in an individual.

30. A method for treating sensitivity to *Lol p* V allergen or an allergen which is immunologically cross reactive with ryegrass pollen allergen in an individual comprising administering similtaneously or sequentially to said individual a therapeutically effective amount of at least two compositions of claim 23 or 24.

31. A therapeutic composition comprising a pharmaceutically acceptable carrier or diluent and at least two *Lol p* V peptides, selected from the group consisting of peptides, 6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 12 (SEQ ID NO:14), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), 28 (SEQ ID NO:30), 33 (SEQ ID NO:35) and 34 (SEQ ID NO:36) and wherein said composition comprises a sufficient percentage of the T cell epitopes of *Lol p* V such that upon administration of the composition to an individual sensitive to *Lol p* V, T cells of the individual are tolerized to *Lol p* V.

32. A composition of claim 23 comprising a combination of peptides selected from the group consisting of:

14 (SEQ ID NO:16) and 22 (SEQ ID NO:24);

14 (SEQ ID NO:16) and 25 (SEQ ID NO:27);

19 (SEQ ID NO:21) and 22 (SEQ ID NO:24);

19 (SEQ ID NO:21) and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16), 22 (SEQ ID NO:24), and 25 (SEQ ID NO:27);

19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), and 34 (SEQ ID NO:36);

19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), and 34 (SEQ ID NO:36);

14 (SEQ ID NO:16), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 25 (SEQ ID NO:27);

14 (SEQ ID NO:16), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), and 34 (SEQ ID NO:36);

6 (SEQ ID NO:8), 7 (SEQ ID NO:9), 9 (SEQ ID NO:11), 12 (SEQ ID NO:14), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), 33 (SEQ ID NO:35), and 34 (SEQ ID NO:36);

6 (SEQ ID NO:8), 9 (SEQ ID NO:11), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), and 34 (SEQ ID NO:36); and

6 (SEQ ID NO:8), 9 (SEQ ID NO:11), 14 (SEQ ID NO:16), 16 (SEQ ID NO:18), 19 (SEQ ID NO:21), 22 (SEQ ID NO:24), 25 (SEQ ID NO:27), and 34 (SEQ ID NO:36).

33. A use of a composition of claim 31 or 32 for the manufacture of a medicament for treating sensitivity to *Lol p* V allergen or an allergen which is immunologically cross reactive with *Lol p* V allergen in an individual comprising administering to said individual.

34. A method for treating sensitivity to *Lol p* V allergen or an allergen which is immunologically cross reactive with *Lol p* V allergen in

an individual comprising administering to said individual a therapeutic composition of claim 32.

35. A modified peptide of claim 5.

36. A therapeutic composition comprising at least one isolated peptide of claim 42.

```
CGCTATCCCTCCTCGTACAAACAAACGCAAGAGCAGCAATGGCCGTCCAGAAGTACACG      60
                                          M  A  V  Q  K  Y  T
                                        -25            -20

GTGGCTCTATTCCTCGCCGTGGCCCTCGTGGCGGGCCCGGCCGCCTCCTACGCCGCTGAC    120
 V  A  L  F  L  A  V  A  L  V  A  G  P  A  A  S  Y  A  A  D
       -15            -10            -5             1

GCCGGCTACACCCCCGCAGCCGCGGCCACCCCGGCTACTCCTGCTGCCACCCCGGCTGCG    180
 A  G  Y  T  P  A  A  A  A  T  P  A  T  P  A  A  T  P  A  A
       5             10             15             20

GCTGGAGGGAAGGCGACGACCGACGAGCAGAAGCTGCTGGAGGACGTCAACGCTGGCTTC    240
 A  G  G  K  A  T  T  D  E  Q  K  L  L  E  D  V  N  A  G  F
       25             30             35             40

AAGGCAGCCGTGGCCGCCGCTGCCAACGCCCCTCCGGCGGACAAGTTCAAGATCTTCGAG    300
 K  A  A  V  A  A  A  A  N  A  P  P  A  D  K  F  K  I  F  E
       45             50             55             60

GCCGCCTTCTCCGAGTCCTCCAAGGGCCTCCTCGCCACCTCCGCCGCCAAGGCACCCGGC    360
 A  A  F  S  E  S  S  K  G  L  L  A  T  S  A  A  K  A  P  G
       65             70             75             80

CTCATCCCCAAGCTCGACACCGCCTACGACGTCGCCTACAAGGCCGCCGAGGGCGCCACC    420
 L  I  P  K  L  D  T  A  Y  D  V  A  Y  K  A  A  E  G  A  T
       85             90             95             100
```

Fig. 1

SUBSTITUTE SHEET

```
CCCGAGGCCAAGTACGACGCCTTCGTCACTGCCCTCACCGAAGCGCTCCGCGTCATCGCC   480
 P  E  A  K  Y  D  A  F  V  T  A  L  T  E  A  L  R  V  I  A
      105             110             115             120

GGCGCCCTCGAGGTCCACGCCGTCAAGCCCGCCACCGAGGAGGTCCCTGCTGCTAAGATC   540
 G  A  L  E  V  H  A  V  K  P  A  T  E  E  V  P  A  A  K  I
      125             130             135             140

CCCACCGGTGAGCTGCAGATCGTTGACAAGATCGATGCTGCCTTCAAGATCGCAGCCACC   600
 P  T  G  E  L  Q  I  V  D  K  I  D  A  A  F  K  I  A  A  T
      145             150             155             160

GCCGCCAACGCCGCCCCCACCAACGATAAGTTCACCGTCTTCGAGAGTGCCTTCAACAAG   660
 A  A  N  A  A  P  T  N  D  K  F  T  V  F  E  S  A  F  N  K
      165             170             175             180

GCCCTCAATGAGTGCACGGGCGGCGCCTATGAGACCTACAAGTTCATCCCCTCCCTCGAG   720
 A  L  N  E  C  T  G  G  A  Y  E  T  Y  K  F  I  P  S  L  E
      185             190             195             200

GCCGCGGTCAAGCAGGCCTACGCCGCCACCGTCGCCGCCGCGCCCGAGGTCAAGTACGCC   780
 A  A  V  K  Q  A  Y  A  A  T  V  A  A  A  P  E  V  K  Y  A
      205             210             215             220

GTCTTTGAGGCCGCGCTGACCAAGGCCATCACCGCCATGACCCAGGCACAGAAGGCCGGC   840
 V  F  E  A  A  L  T  K  A  I  T  A  M  T  Q  A  Q  K  A  G
      225             230             235             240
```

Fig. 1 cont.

```
AAACCCGCTGCCGCCGCTGCCACAGGCGCCGCAACCGTTGCCACCGGCGCCGCAACCGCC    900
 K  P  A  A  A  A  A  T  G  A  A  T  V  A  T  G  A  A  T  A
     245              250              255              260

GCCGCCGGTGCTGCCACCGCCGCTGCTGGTGGCTACAAAGCCTGATCAGCTTGCTAATAT    960
 A  A  G  A  A  T  A  A  A  G  G  Y  K  A  *
     265              270              275

ACTACTGAACGTATGTATGTGCATGATCCGGGCGGCGAGTGGTTTTGTTGATAATTAATC    1020

TTCGTTTTCGTTTCATGCAGCCGCGATCGAGAGGGCTTGCATGCTTGTAATAATTCAATA    1080

TTTTTCATTTCTTTTTGAATCTGTAAATCCCCATGACAAGTAGTGGGATCAAGTCGGCAT    1140

GTATCACCGTTGATGCGAGTTTAACGATGGGGAGTTTATCAAAGAATTTATTATTAAAAA    1200

AAAAAAAAAAAAAAAAAAAAAAAAAAAAA                                   1229
```

Fig. 1 cont.

PEPTIDE NO.	PEPTIDE SEQUENCE N-TERMINUS / C-TERMINUS
1	ADAGYTPAAAAT
2	AAATPATPAATPA
3	AATPAAAGGKAT
4	GKATTDEQKLLE
5	KLLEDVNAGFKA
6	GFKAAVAAAANA
7	AANAPPADKFKI
8	KFKIFEAAFSES
9	FSESSKGLLATS
10	LATSAAKAPGLI
11	PGLIPKLNTAYD
12	TAYDVAYKAAEG
13	AAEGATPEAKYD
14	AKYDAFVTALTE
15	ALTEGLRVIAGA
16	IAGALEVHAVKPA
17	AVKPATEEVPAA
18	VPAAKIPTGELQI
19	GELQIVDKIDAA
20	IDAAFKIAATAA
21	ATAANAAPTNDK
22	TNDKFTVFESAF
23	EASFNKALNECT
24	NECTGGAYETYK
25	ETYKFIPSLEAA
26	LEAAVKQAYAAT
27	YAATVAAPEVKY
28	EVKYAVFEAALT
29	AALTKAITAMTQA
30	AMTQAQKAGKPA
31	GKPAAAAATAAA
32	TAAATVATAAAT
33	AAATAAAGAATA
34	GAATAAAGGYKA

Fig. 2

SUBSTITUTE SHEET

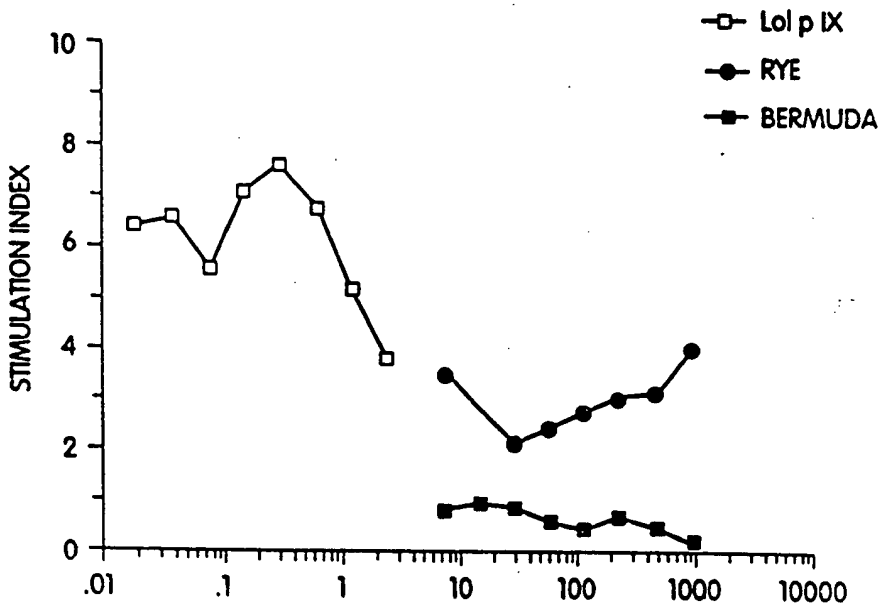
Lol p IX
RYE
BERMUDA
STIMULATION INDEX
10
8
6
4
2
0
.01
.1
1
10
100
1000
10000

Fig. 3

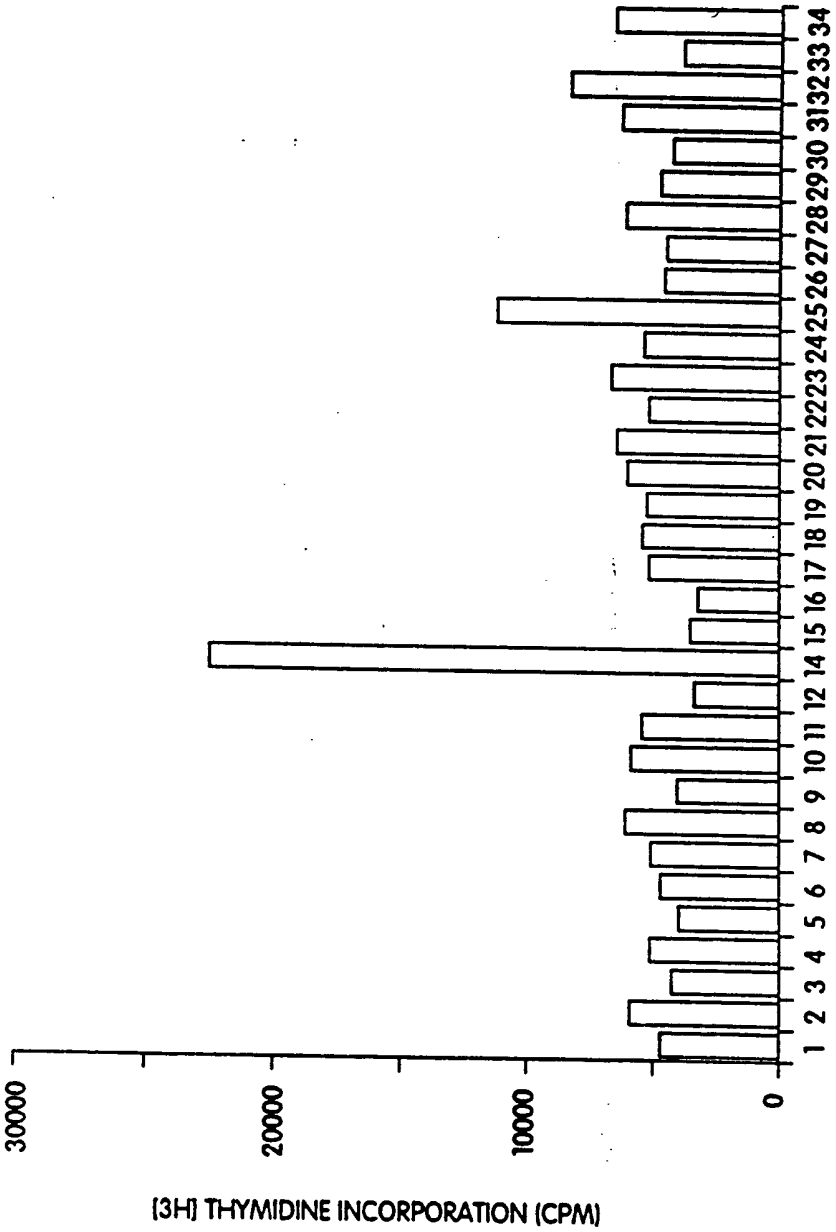
30000
20000
10000
0
[3H] THYMIDINE INCORPORATION (CPM)
1 2 3 4 5 6 7 8 9 10 11 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
PEPTIDE

Fig. 4

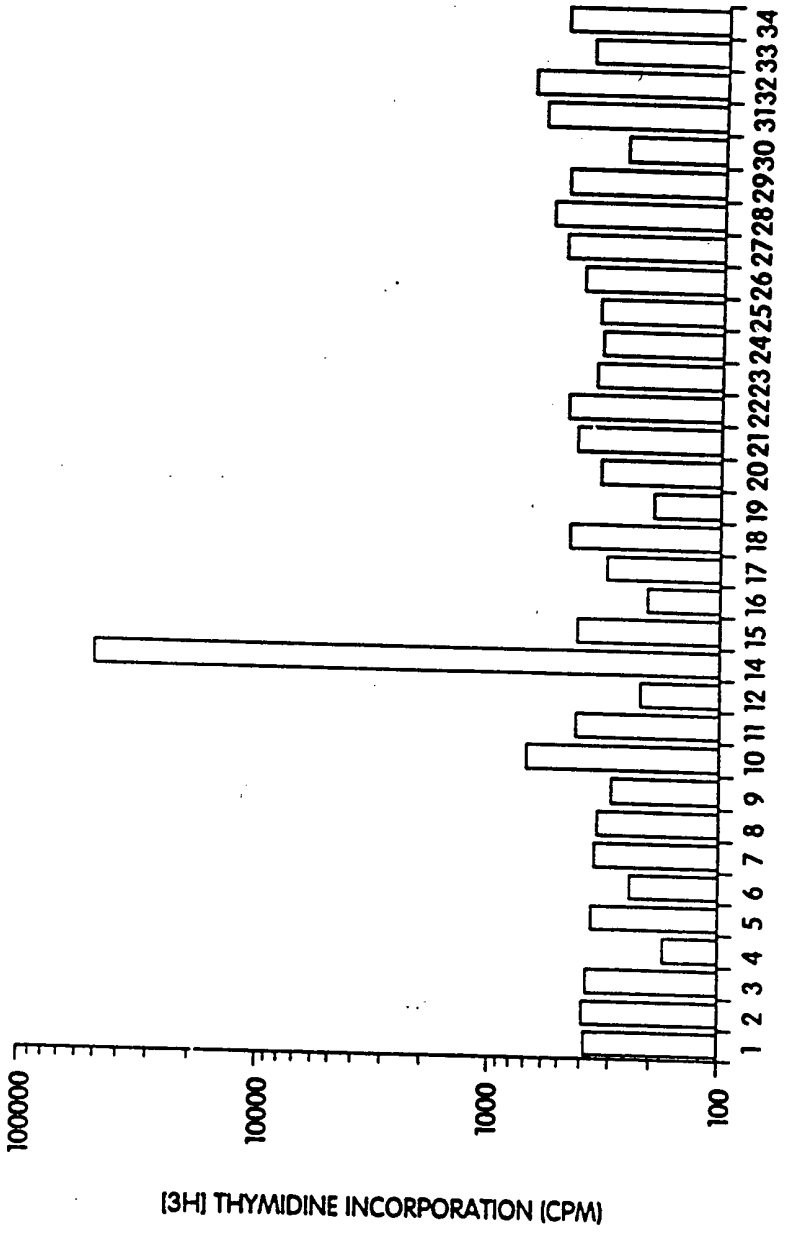
100000
10000
1000
100
1 2 3 4 5 6 7 8 9 10 11 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
PEPTIDE
[3H] THYMIDINE INCORPORATION (CPM)

Fig. 5

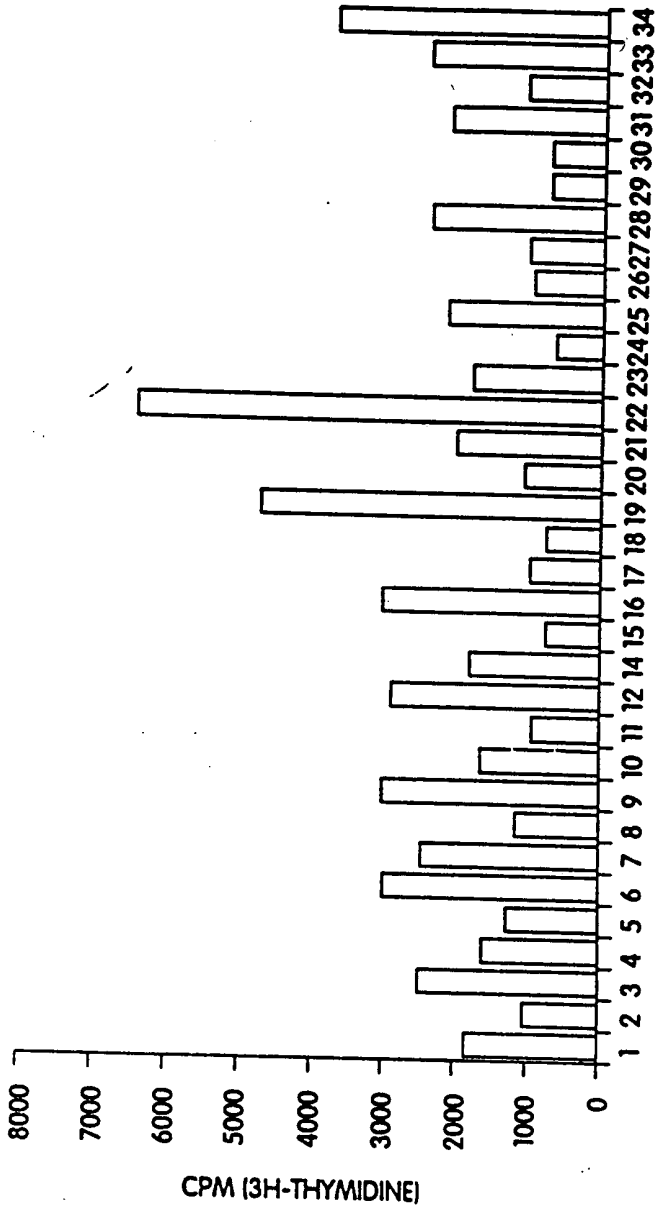
8000
7000
6000
5000
4000
3000
2000
1000
0
CPM (3H-THYMIDINE)
1 2 3 4 5 6 7 8 9 10 11 12 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34
PEPTIDE

Fig. 6

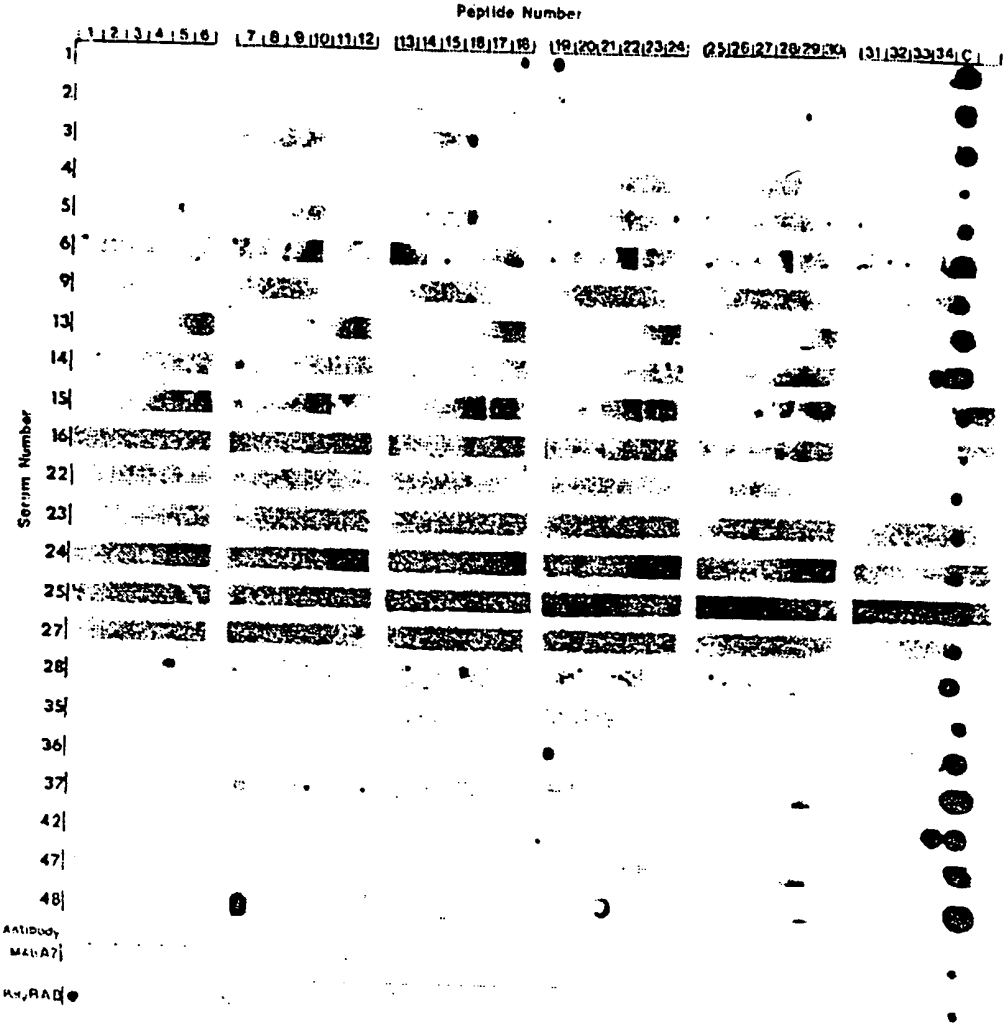

Fig. 7

SUBSTITUTE SHEET

Fig. 8A

PEPTIDE NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	C
(a) SERUM NO.																																			
1																		5	11															11
3																4																			11
5					2											4						2		2											12
6										7			4																						11
9																2																			12
13																								3						2					13
14							2																										11		12
15																9								5			2			4					11
16																													3						5
24					2	2																	10							1					12
25																2								2	3										12
27																								2											12
28					5		1									3											2		2						13
36																			11																13
42																																	13		13
48							11															12													13
C1																																			2
C2																																			1
SCORE/PEPTIDE	0	0	0	0	9	2	15	0	0	7	0	0	4	0	0	24	0	5	22	0	12	2	10	14	3	0	4	0	5	14	0	0	0	24	
NO. OF +VE SERA	0	0	0	0	1	1	3	0	0	1	0	0	1	0	0	6	0	1	2	0	1	1	1	5	1	0	2	0	2	3	0	0	0	2	
SCORE/SERA	0	0	0	0	3.0	2.0	5.0	0	0	7.0	0	0	4.0	0	0	4.0	0	5.0	11.00		12.0	2.0	10.0	2.8	3.0	0	2.0	0	2.5	4.7	0	0	0	12.0	

MAb A7							2												2					2	2				2				2	12
MAb A1				2	3	2	2	2			2				2		2	2			3	3	4		2		3							12
POLY RAB	7						2					2				2		3					2						2	2			2	8
SCORE/PEPTIDE	7	0	0	2	3	2	6	2	0	0	0	2	2	0	0	2	2	2	7	0	0	3	3	1	2	2	0	3	2	4	0	0	0	4	
NO. OF +VE SERA	1	0	0	1	1	1	3	1	0	0	0	1	1	0	0	1	1	1	3	0	0	1	1	3	1	1	0	1	1	2	0	0	0	2	
SCORE/SERA	7.0	0	0	2.0	3.0	2.0	2.0	2.0	0	0	0	2.0	2.0	0	0	2.0	2.0	2.0	2.3	0	0	3.0	3.0	2.7	2.0	2.0	0	3.0	2.0	2.0	0	0	0	2.0	

Fig. 8B

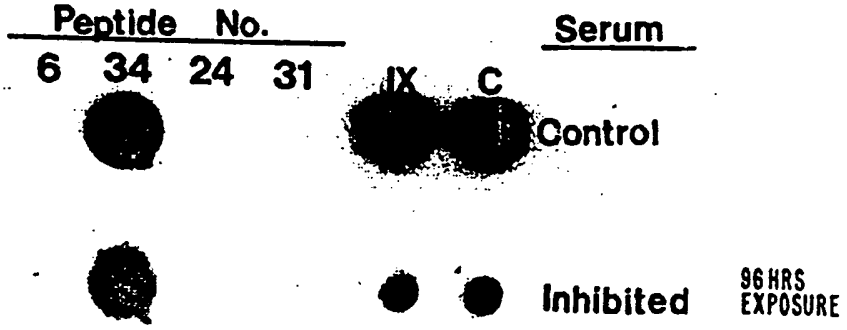
Peptide No.
6
34
24
31
IX
C
Serum
Control
Inhibited
96 HRS EXPOSURE

Fig. 9

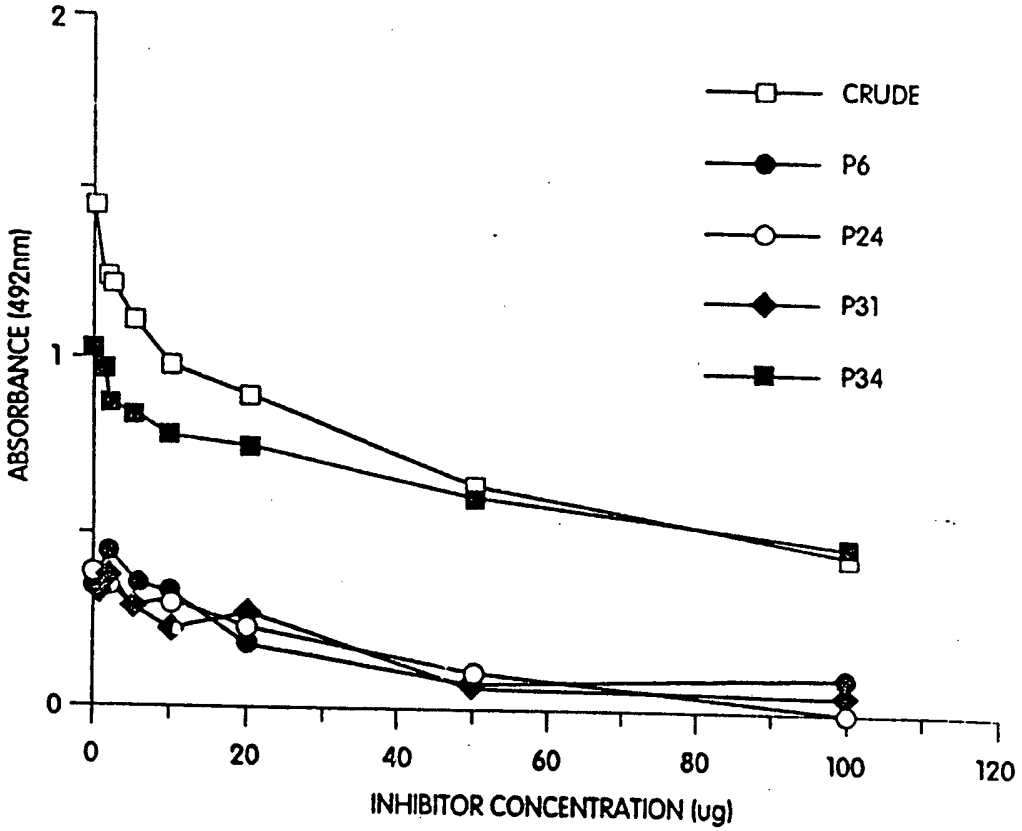
2
1
0
ABSORBANCE (492nm)
0
20
40
60
80
100
120
INHIBITOR CONCENTRATION (ug)
CRUDE
P6
P24
P31
P34

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 93/00415

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.[5] C07K 7/08; C12N 15/29; C12P 21/02, 21/08; G01N 33/68; A61K 39/36, 37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: C07K 7/08

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base, and where practicable, search terms used)
WPAT (DERWENT DATABASE) KEYWORDS: RYE GRASS OR LOLIUM PERENNE
CASM (CHEMICAL ABSTRACTS) KEYWORDS: (RYE GRASS OR LOLIUM PERENNE) AND (ALLERGEN OR POLLEN OR ANTIGEN); LOL P

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	AU,A,28244/92 (The University of Melbourne) 25 March 1993 (25.03.93) Claims 1-4	9,10
X	AU,A,84083/91 (The University of Melbourne) 5 March 1992 (05.03.92) pages 9-10, 12, 14-15, 32-33 and claims 60 and 66	1-36
Y		1-5,12,13
X	AU,A,31644/89 (The University of Melbourne) 28 September 1989 (28.09.89) claims 1-4	9,10

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 November 1993 (23.11.93)	26 NOV 1993 (26.11.93)
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. (06) 2853929	Authorized officer G. PETERS Telephone No. (06) 2832242

Form PCT/ISA/210 (continuation of first sheet (2)) (July 1992) copljm

INTERNATIONAL SEARCH REPORT
Information on patent family membe.

International application No.
PCT/AU 93/00415

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
AU 84083/91	CA 2089735 WO 92/03550
AU 31644/89	DK 2281/90 EP 406286 WO 89/09260 GB 9127140 WO 93/13350
AU 24409/92	WO 93/04174

END OF ANNEX

INTERNATIONAL SEARCH R' 'ORT
Information on patent family membe.

International application No.
PCT/AU 93/00415

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpos' of information.

Patent Document Cited in Search Report	Patent Family Member
AU 84083/91	CA 2089735 WO 92/03550
AU 31644/89	DK 2281/90 EP 406286 WO 89/09260 GB 9127140 WO 93/13350
AU 24409/92	WO 93/04174

END OF ANNEX

Form PCT/ISA/210(patent family annex)(July 1992) eopljm